APPRAISAL OFANCIENT INSIGHT LEADING TO MODERN ON *Trichosanthes cucumerina*Linn LEAVES OFFERS IMMENSE SCOPE TO IMPROVE R & D LANDSCAPE OF CARDIO PROTECTION AND WOUND HEALING

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Chennai-600 032

In partial fulfilment of the requirements
for the award of the degree of

MASTER OF PHARMACY
IN
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Submitted by

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DEPARTMENT OF PHARMACOGNOSY

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CHAPTER-1

INTRODUCTION

CHAPTER - 1

INTRODUCTION

Medicinal plants from ancient times to the present

Plants have been used in treating human diseases for thousands of years. Some thousands of years ago, it appears man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found. One of these allegedly ancient medicinal herbs, yarrow, is discussed in this work as a modern medicinal plant.

Since prehistoric times, shamans or medicine men and women acquired a tremendous knowledge of medicinal plants.

Up until the 18th century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16th century Italy, in Pisa, Padova and Florence, were medicinal plant gardens attached to medical faculties or schools.The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines.

Herbal medicines versus pharmaceuticals

Two classes of use of medicinal preparations are commonly recognized, often under the titles herbal and pharmaceutical. Pharmaceuticals, discussed below, are refined or synthesized drugs. The World Health Organization has defined medicinal herbals as follows (WHO 1996):

"Finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof,

whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substances of this nature. Herbal medicines may contain excipients [inert additives such as starch used to improve adhesive quality in order to prepare pills or tablets] in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constituents of plants, are not considered to be herbal medicines. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin

Herbals in India: Quality, Safety and Efficacy

Herbal medicines are being used in India since immemorial and it has been documented in Veda. Herbal medicines are also used since long back in different countries like China. In India, herbal medicines are being used in Ayurveda, Siddha, and Unani system of medicines. Ayurvedic system is being practiced since 6000 B.C., and Chinese herbal medicines since 5000 B.C., whereas the modern system of medicines started since 1800 A.D.

This was popular may be because of the experience and abundant availability of medicinal plants in India due to its varied agro climatic zones. India has around 45,000 species of plants, out of which 15,000-20,000 plants have proven medicinal value. The traditional system utilizes around 7,000-7,500 species in its formulations. Ayurveda uses 2000, Siddha 1300, Unani1000, and others like Tibetan in the modern medicine.

According to the World Bank report, herbal medicines has a global market of US$ 80 - 100 billion and this market is expected to reach US$ 2500

billion by the year 2010 and US$ 5 trillion by the year 2050. The Indian herbal drug market is about $ 1 billion and the export of herbal crude extract is about $ 80 million. We have about 7800 manufacturing units engaged in manufacturing of herbal drugs in India, which are consuming 200 tonnes of herbs annually (Mandal SC, 2012).

Growing market trend & healthcare concerns of herbal supplements

In the U.S, the use of herbal supplements is continuously growing, with over five billion dollars spent on herbals in 2010. It is estimated that over 40% of Americans are using some form of herbal product, also known as complementary and alternative medicine (CAM). In 2010, the American Botanical Council released a statistical report on herbal supplement sales (turnover in increasing order)

Herbal supplement	US Dollar sales
Ginseng	$7,283,017
St. John's wort	$8,871,864
Black cohosh	$9,303,047
Milk thistle	$11,266,790
Echinacea	$12,822,940
Ginkgo	$15,017010
Garlic	$16,976,220
Soy	$16,984,640
Saw palmetto	$18,839,780
Cranberry	$35,806,000

Media plays a major role for increasing usage of herbals in U.S. Peoples have access to the Internet and television allowing them to "self- treat" without physician's consultation. To many patients, herbal supplements offer a more convenient treatment option compared with prescription medications. Herbal products are available without a prescription and allow the patient to remain

anonymous without having to see a physician or another healthcare provider. Moreover herbal medicines are economical than allopathic medicines.

The major drawback of herbal supplements is quality control studies. Difficult to evaluate randomized, control trials. On the other hand different solvent using for preparation of extracts, difficult to determine therapeutic equivalence among the herbal products.

The efficacy of herbal supplements is difficult to determine because large, randomized, controlled trials are lacking. In the literature, clinical trials on herbal supplements often use different extracts, making it impossible to determine equivalence among the herbal products being studied. Therefore, the pharmacological response and effectiveness of an herbal supplement is difficult to determine. Some commonly used herbal product and its adverse effect given below.

Commonly used Herbal Products and Their Indication and Adverse Effects

Common name	Latin name	Indication	Adverse effects
Ginseng	*Panax ginseng*	Memory, cancer, erectile dysfunction, diabetes	Diarrhoea, headache, nausea, vomiting, insomnia, hypertension, estrogenic activity, vaginal bleeding
Garlic	*Allium sativum*	Dyslipidemia	GI, heartburn, allergic reactions, excessive bleeding
Echinacea	*Echinacea purpurea*	Upper Respiratory Tract Infections	Nausea, vomiting, dizziness, dyspnoea, angioedema
Ginkgo	*Ginkgo biloba*	Cognitive impairment, dementia, peripheral vascular diseases, hypertension	GI, headache, nausea, vomiting, allergic reactions, prolonged bleeding

As healthcare professionals, it is important to determine safety concerns of herbal supplements and the potential for significant drug-herbal interactions.

Adverse reactions also associated with the use of herbal supplements are important issues that many people are unaware exist. Many consumers think the word "natural" on the product label meant to safe and free of harmful side effects.

The potential for significant drug- herbal interactions is of great concern in patients while taking prescription medications and can have serious detrimental effects on patient care. Often, consumers of herbal supplements also have multiple co-morbidities such as hypertension, diabetes and dyslipidemia, and may consume a greater number of prescription medications, increasing the potential for drug interactions. Patients may not be interest to voluntarily share this information with their primary care physician, so pharmacist and all healthcare providers to take the time to obtain the patients' complete medication history, including prescription medications and over-the- counter products such as herbals, dietary supplements, vitamins, and minerals.

Many drug interactions can be dangerous and have a significant impact on patients' health. For example, herbal supplements such as ginkgo, ginseng, garlic and St. John's Wort, may potentially interact with warfarin (Coumarin®), an anticoagulant medication. This drug-herbal interaction leads to increase risk of acute bleeding. Ginkgo and garlic are proven as an anti-platelet activity and may increase the chance for bleeding when taken with other medications such as aspirin and non- steroidal anti-inflammatory drugs (NSAIDs).

Drug Interactions of Common Herbal Products

Although the exact mechanisms are currently unknown, ginkgo has shown in case reports to significantly alter the therapeutic effects of thiazide diuretics and trazodone (Oleptro), a serotonin agonist/antagonist used to treat depression. Ginkgo may decrease the metabolism of trazodone and therefore increase sedation, which is a major substrate of the cytochrome P450 (CYP450) 3A4 isoenzyme. Ginkgo is having hypertensive properties that may diminish the antihypertensive effects of thiazide diuretics, such as hydrochlorothiazide (Microzide). Other potential drug-herbal interactions associated with ginkgo include phenytoin, valproic acid, nifedipine, propranolol, alprazolam, midazolam, cyclosporine, digoxin, haloperidol, proton-pump inhibitors (PPIs), and sulfonylureas. Ginseng contains ginsenosides metabolised by cytochrome P450 (CYP450) isoenzyme that alter the metabolism of many drugs. Ginseng increases metabolism of loop diuretics, calcium channel blockers, monoamine oxidase inhibitors, digoxin and reduces its effects. Ginseng contraindicated in patients receiving estrogen therapy which increase the chance of breast nodules. Garlic increases the metabolism of protease inhibitors like Ritonavir, Saquinavir (substrate for CYP450 3A4 isoenzyme). Co-administration of garlic supplement and protease inhibitors is not advised. A more extensive list of drug- herbal interactions is presented in the following table

Herbal supplement	Drug-herbal interactions
Ginseng	Warfarin, furosemide, nifedipine, phenelzine, digoxin, NSAIDs, MAOIs, antidiabetics, antiestrogens
Ginkgo	Warfarin, aspirin, NSAIDs, thiazide diuretics, phenytoin, valproic acid, nifedipine, propranolol, alprazolam, midazolam, cyclosporine, digoxin, haloperidol, PPIs, sulfonylureas, trazodone
St. John's wort	Warfarin, digoxin, oral contraceptives, HIV medications, epilepsy medications, cyclosporine, simvastatin, MAOIs, SSRIs, TCAs, opioid analgesics
Garlic	Warfarin, aspirin, NSAIDs, protease inhibitors
Echinacea	Immunosuppressant, CCBs, protease inhibitors, cyclosporine

The use of herbal supplements has continued to grow over the last decade with some of the most common products, including ginkgo, ginseng, garlic, Echinacea, and St. John's wort claiming to treat conditions including dementia, hypertension, diabetes, dyslipidemia, respiratory infections, and depression. It is the responsibility of healthcare providers to review all patient medications and to educate patients on the important information that many product labels omit in order to provide optimal patient care (Crosby BL, 2012).

If we can adopt certain life styles & dietary controls, the effect of herbals can be enormously increased. Some useful tips for safe & effective use of herbals are given below:

1. Take herbal drugs under a physician's direction.
2. Do not take supplements containing the amino acids phenylalanine or tyrosine along with Mono Amine Oxidase Inhibitors (MAOI). Also avoid the co-administration of artificial sweetener aspartame with herbal products.
3. Avoid smoked or processed meats.

4. Individuals with high blood pressure should avoid excess salt, eat more fruits, vegetables with potassium and fat-free or low-fat dairy products, Herbal and traditional medicine adhere more to a healthy lifestyle. Finally, laughing provides us with the natural inner massage, relives stress and through change of mood up to 30% of cure!

Recent scientific investigations had confirmed the efficacy of many of the plants and plant formulations, some of which were remarkably effective. Those herbs that appear more effective and relatively non-toxic have substantially documented. Use of herbal medicine is a practice to adopt a healthy life style. Because of increased value and absence of suitable alternatives in modern medicine, even the physicians practicing modern medicine in developed countries frequently prescribe herbal medicines (Mathew M, 2012). Many patients suffer from chronic disease like cancer, cardio vascular diseases, diabetes are not well catered by modern medicine. Patients with chronic disease generally prefer herbals than modern medicine which is cause severe adverse effect. On the other hand naturalness of herbals denotes that these are safe (Ritter JM, 2008). Now it is the need of the hour to find out a safe drug lead to treat chronic diseases like cardio vascular disease, diabetes, and cancer etc.

Cardiovascular diseases (CVDs)

CVDs are the number one cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke. Low- and middle-income countries are disproportionally affected: over 80% of CVD deaths take place in low- and middle income countries and occur almost equally in

men and women. By 2030, almost 25 million people will die from CVDs, mainly from heart disease and stroke. These are projected to remain the single leading cause of death. Most cardiovascular diseases can be prevented by addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, raised blood pressure, diabetes and raised lipids. 7.5 million Deaths each year, or 13% of all deaths can be attributed to raised blood pressure. This includes 51% of deaths due to strokes and 45% of deaths due to coronary heart disease. There are also a number of underlying determinants of CVDs, or "the causes of the causes". These are a reflection of the major forces driving social, economic and cultural change – globalization, urbanization, and population ageing. Other determinants of CVDs include poverty, stress and hereditary factors. There is a need for increased government investment in prevention and early detection through national programmes aimed at prevention and control of non communicable diseases including CVDs (WHO Report, Sep 2012).

Number of therapies available for hypertension including β-blockers, α-blockers, diuretics, ACE inhibitors, calcium channel blockers. Each category work differently among them. All of them having numerous side effect like β-blockers (bradycardia, depression, dizziness, confusion, fatigue), α-blockers (orthostatic hypotension, dizziness, fainting, tachycardia, arrhythmia, drowsiness), diuretics (hypokalemia, hyperuricemia, hyperglycaemia, anorexia), ACE inhibitors (dizziness, fainting, light headness, insomnia, dry cough, fatigue), calcium channel blockers (bradycardia, dizziness, fainting, flushing) (Goodman & Gilman, 2011).

Many plants proven as a cardio tonic due to its antioxidant constituents. Plants like *Terminalia arjuna*, *Allium sativum*(Garlic), *Allium cepa* (Onion), *Asparegus*

racemosus, *Cassia fistula*, *curcuma longa* (Turmeric), *Embilica officinalis* (Amla), *Ocimum santum* (Tulsi), *Phyllanthus amarus*, *Vitis vinifera* (Grapes), *Withania somnifera* (Aswaganda), *Zingiber officinalis* (Ginger) shows cardio tonic activity. Antioxidant and free radical (Hydroxyl radicals, Super oxide anion, Thiyl radicals, Total ROS, Peroxyl radicals, Singlet oxygen) scavenging property mainly responsible for cardio protective activity of medicinal plants (Tilak Jain JA, Devasagayam TPA, 2006).

Wound management is an important health burden on the community (Arana V *et al*, 2004). Chronic wound have a very large social and quality of life impact on individuals and carers. (Williams JZ, Barbul A, 2003). Essential role is played by nutrition in wound healing and hence nutritional support need to be considered as a fundamental part of wound management. It is also cost effective. More over nutrition may delay healing and impair wound strength and prone to break down (Crowe T, Brock bank C, 2009).Wounds may arise from a variety of aetiologicals such as venous hypertension, neuropathy (e.g. diabetes mellitus), Peripheral aetrial disease, vasculitis, burns (Rashid M *et al*, 2009). A chronic wound takes more than 4 to 6 weeks to heal. Nutrition like protein, amino acid, carbohydrates, and minerals supports healing process.

Previous reports suggested CVD as a consequence of the other chronic disease diabetes. Diabetes is a major burden in the healthcare system. Patients suffering with these diseases have to take medication separately for this complication. So far no medicines available to treat all this chronic disease simultaneously. Research focussed towards safe, drug leads with the above aim possibly derived from natural source e.g.

medicinal plants. Searching medicinal plants effective on diabetes and its associated complication such as CVD, diabetic foot ulcer, etc will be most effective research.

Reason for selection of *Trichosanthes cucumerina* :

A few of genera are economic sources of food and widely used in traditional medicine, agriculture and industry. These genera received a great level of scientific interest as they contain medicinally important secondary metabolites possessing useful biological activities. ***Trichosanthes cucumerina*** provide advantage as a profitable food crop for producing fruits. The exceptional food and medicinal value of this plant has long been recognized and economically the genus is of appreciable importance as a source of edible fruit; The ***Trichosanthes cucumerina*** is popularly known as snake gourd in English, belongs to the family Cucurbitaceae is known Pudal in Tamil, It has become naturalised in the several parts of South Asia, East Africa and is grown in South India on a large scale and in North India cultivation more or less restricted to kitchen gardens but gaining popularity now.(Anonumous 2005). The leaves of *Trichosanthes cucumerina* really do not have any match as a cheap natural and easily available plant. It is traditionally known to be useful for the treatment of wide panel of diseases like diabetes,wounds,skin diseaeses, alopecia, diarrhoea, haematuria, malaria, bronchitis, refrigerant, liver diseases etc. Antihepatotoxic, antifertility, hepatoprotective activity, anti-inflammatory, antidiabetic, antiulcer and antibacterial, diuretic, antioxidant, antihistamine, gastroprotective activities have been studied and found effective. Leaf is used as cardiotonic, anti pyretic, antiperiodic, emetic, antihelmintic, and externally applied over bald patches of alopecia. Juice is rubbed over the liver in liver congestion and all over the body in remittent fever. Hair growth promoting activity, anti bacterial activity, larvicidal activity and toxicity studies have been performed and found

effective and safe. Fruit used as an antioxidant, anti-inflammatory, antibacterial, anticariogenic, antifungal, hypoglycemic, anti diabetic and excellent source of fibres, vitamins, minerals and proteins. Anticancer activity, cardioprotective activity, antioxidant activities and toxicity studies have been investigated and found effective and safe. Seed is used as cooling, as anthelmintic, antidiarrhoeal, abortifacient, aphrodisiac, astringent, febrifuge. Cardioprotective, antioxidant, antibacterial, antispasmodic, insecticidal, antidiabetic, anti febrile, actions have been studied. Roots useful in treating diabetes, skin swelling,convulsion. Anticancer activity was carried out and found effective. This plant is much more popular in India and widely cultivated. In India fruits used as food and is rich source of nutrition constituted with proteins, fat, fibre, carbohydrates, vitamin A & E, ascorbic acid, carotenoids, lycopene,flavonoids, carotenoids, phenolic acids and minerals like potassium, phosphorous, magnesium, zinc etc. The economic aspect of this crop evidently proved that as commercial crop. In fact the revenue generated by this crop can be further magnified by many folds, if its medicinal applications are scientifically explored well.

The review of literature reveals that researches have to be carried out to bridge the lacunae between ethnopharmacological uses and its validation.

The present study assesses the potential of *Trichosanthes cucumerina* in relation to its traditional uses and in terms of findings based on modern bio scientific research.

CHAPTER -2

LITERATURE REVIEW

CHAPTER - 2

LITERATURE REVIEW

Scientific classification of *T.cucumerina* (Sandhya *et al.,* 2010)

Kingdom: Plantae

Division : Magnoliophyta

Class : Mangnoliophyta

Order : Cucurbitales

Family : Cucurbitaceae

Genus : *Trichosanthes*

Species : *Cucumerina*

Vernacular names: (Anonymous 2005).

Sans and Hindi : Chachinda

Beng : Chichinga

Mar : Padwal

Guj : Padavali

Tel : Lingapotla

Tam : Pudalai

Kan : Padavalakayi

Mal : Patavalanga

Oriya : chhachhindara

WHOLE PLANT

ETHNOMEDICAL INFORMATION

T.cucumerina is used as anti-diabetic, gastroprotective, anti-inflammatory, anti-oxidant, lipid lowering activities and showed non-toxicity in rodents. It is

useful for the treatment of wounds including boils, sores, skin eruptions such as eczema and dermatitis.[Arawwawala LD *et al.,* 2011]

T.cucumerina whole plant used as anti-fertility, anti-bacterial, hepatoprotective, anti-inflammatory, gastroprotective activity.[Vijay Kumar R *et al.,* 2012]

T.cucumerina is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious boils, acute colic, diarrhoea, haematuria and skin allergy.[Joji RL *et al.,* 2010, Sandhya S *et al.,* 2012, Sagar L *et al.,* 2012]

T.cucumerina is used as abortifacient, vermifuge, refrigerant, purgative, malaria, laxative, hemagglutinant, emetic, cathartic, bronchitis and anthelmintic, hydragogue, stomachic.[Nadkani KM., 2002, Sandhya S *et al.,* 2012, Linn TK., 2012, Sagar L *et al.,* 2012]

T.cucumerina is one among the many constituents in various Ayurvedic formulations used for the treatment of liver disorders and other diseases.[Linn TK., 2012]

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PHARMACOGNOSTICAL STUDIES

Genus of *T.cucumerina* is found wild throughout the Southern and Eastern Asia, Australia and Islands of the Western Pacific. It was probably domesticated in ancient times in India.[Anonymous ., 2002]

T.cucumerina is a newly introduced crop of increasing importance in several parts of Africa, including Ghana and Nigeria. *Trichosanthes* genus comprises about 100 species, of which a few (snake gourd) have been domesticated in Asia.

T.cucumerina distinguished within two varieties(*var.cucumerina &var.anguina*).Wild *var.cucumerina* cultivated in India, SriLanka and China, through South-East Asia, to northern Australia and the *var.anguina* cultivated in West and Central Africa.[Khare CP., 2007]

T.cucumerina is a monoecious annual herb climbing by 2-3 branched tendrils upto 5 to 6 meters high or less. [Sandhya S *et al.,* 2010]

In Africa, *T. cucumerina* is locally grown as a vegetable in home gardens but in many countries of tropical Asia *T.cucumerina* grown as a minor vegetable. Commercial growers around big cities in East Africa occasionally grow cultivars of snake gourd imported from India for people of Indian origin. It is also reported from India through Malaya to tropical Australia. [Sandhya S *et al.,* 2010]

T.cucumerina contains rich source of proteins, fat, fibres, carbohydrates and vit A & E.).[Sagar L *et al.,* 2012]

PHYTOCHEMICAL STUDIES

T.cucumerina contains triterpenes which are

- 23, 24-dihydrocucurbitacin D,
- 2324-dihydrocucurbitacin B,
- Cucurbitacin B,
- 3β-hydroxy-olean-13(18)-en-28-oic acid,
- 3-oxo-olean-13(18)-en-30-oic acid and the
- Sterol 3-*O-β-D*-glucopyranosyl-24ξ-ethyl-cholest-7,
- 22-dien-3β-ol.

The percentage of free fatty acid and acid value were low suggesting increased stability and usefulness in nutritional and industrial applications. [Jiratchariyakul W and Frahm AW., 1992]

Major active constituents of the *T.cucumerina* are triterpenoid and saponins viz., cucurbitacins. [, Kirana H, Srinivasan B., 2008]

Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, steroids, saponins and tannins in the *T.cucumerina* extract. [Arawwawala M *et al.,*2009]

The ethanolic extract of *T.cucumerina* (whole plant) was showed the presence of alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols, tannins. . [Devendra *et al.,* 2009] [a]

Different extracts of (petroleum ether, chloroform, ethanol & distilled water) *T.cucumerina* (whole plant) was revealed the presence of alkaloids, flavonoids, cardiac glycosides, phenols, sterols, tannins, saponins and lignans. .[Devendra *et al.,* 2009][b]

T.cucumerina plant contains carotenoids, flavonoids, lycopene, phenolics and β carotene. [Joji RL *et al.,* 2010]

T.cucumerina extracts (HWE & CEE) contain tannins, saponins, flavonoids and alkaloids as major chemical constituents. [Arawwawala LD *et al.,* 2011]

The preliminary phytochemical analysis of *T.cucumerina* showed the presence of flavonoids, saponins, carbohydrates, terpenoids and alkaloids. . [Raama Murthy *et al.,* 2012]

Methanol extract of *T.cucumerina* contains phenolic compounds, flavonoids and carotenoids. [Joji RL and Beena J., 2013]

PHARMACOLOGICAL ACTIVITY

LD_{50}

LD_{50} value of Aqueous extract of *T.cucumerina* in acute toxicity study was found to be 1.12g/kg bw in mice. [Kirana H, Srinivasan B., 2008]

ACUTE TOXICITY

The methanolic extract of *T.cucumerina* (whole plant) showed no mortality upto a dose level of 2000mg/kg b.w.p.o upto 72 hours. [Sathesh Kumar S *et al.,* 2007]

Oral administration of ethanolic extract of *T.cucumerina* (200&400 mg/kg b.w 7 days) in female albino rats showed no deaths & no change in animals (behaviour, skin effects, loss of hair, defecation or other physiological activities. [Devendra *et al.,* 2009] [a]

CHRONIC TOXICITY

Ethanol extract of (whole plant) *T.cucumerina* was studied (200&400 mg/kg b.w p.o) in female albino rats showed no change in body weight (heart , liver , kidney, lung , spleen, adrenals , esophages , stomach &large intestine) when compared with control. . [Devendra *et al.,* 2009] [a]

ANTI-HEPATOTOXIC ACTIVITY

97% methanolic extract (whole plant) TCME (250mg/kg&500mg/kg b.w/oral) was studied for the anti-hepaotoxic activity on carbon tetra chloride induced (1ml/kg of 50%CCl_4 dissolved in olive oil by s.c) liver damage in wistar albino rats. It significantly reduced the AST , ALT, ALP and TB levels and increase the protein levels in the liver when compared with standard(silymarin 100mg/kg b.w.p.o).The histopathological studies showed decreased necrotic zone and hepatocellular degeneration supported the anti-hepatotoxic activity of TCME.[Sathesh Kumar S *et al.,* 2007]

HEPATOPROTECTIVE ACTIVITY

Pre treatment of methanolic extract of *T.cucumerina* (whole plant)has showed hepatoprotective activity(250&500mg /kg b.w.p.o)on carbon tetrachloride induced hepatotoxicity in rats .It significantly controlled the raise of ALT, AST, ALP, TB and MDA. The TP, ALB and GSH levels were significantly increased. The pre treatment showed decreased necrotic zones and hepatocellular degeneration when compared to the CCl_4 intoxicated liver. This results support to traditional medicine for treatment of liver disorders. [Satheesh K *et al.,* 2009]

ANTI FERTILITY ACTIVITY

Oral administration of ethanolic extract of *T.cucumerina* (whole plant) was showed anti fertility activity (200 & 400mg/kg b.w) in female albino rats. The extract was significantly increased estrous and metaestrous phases , serum regressing follicles(stage IA, IB, IIA IIB), cholesterol level &decreased diestrus and proestrus phases , healthy follicles(class I-IV), corpora lutea , protein, glycogen ,

serum FSH, LH level(RIA) & the enzyme activities (3b-HSD& 17B-HSD)were significantly inhibited in the ovary of rats.[Devendra *et al.,* 2009][a]

ANTI INFLAMMATORY

Hot water extract of *T.cucumerina* (500, 750, 1000 mg/kg)was showed anti-inflammatory activity in wistar rats(carrageenan induced paw oedema).Significant ($p \leq 0.05$)inhibition of the inflammation(750 mg/kg at 5hr)was compared with standard(Indomethacin).This results support to the treatment of inflammatory conditions in Sri Lankan traditional systems of medicine.[Arawwawala M *et al.,* 2010][b]

ANTI-DIABETIC ACTIVITY

Oral administration of aqueous extract(100mg/kg/day)of *T.cucumerina* showed anti-diabetic activity in non- insulin dependent diabetes mellitus in neonatal rat model(STZ 90mg/kg/i.p).*T.cucumerina* has significantly($p<o.o1$)reduced the postprandial blood glucose(2hr)and increase in tissue glycogen content(liver 62% &skeletal muscle 58.8%).Decrease in blood glucose may be due to cucurbitacin glycosides present in the drug.[Srinivasan B, Kirana H., 2008]

ANTI-ULCER ACTIVITY

Oral administration of 50% ethanolic extract of (300, 500 & 800mg/kg) patol churna (ayurvedic formulation of *T.cucumerina*) was showed anti-ulcer activity in wistar rats against ethanol, aspirin and pylorus ligated gastric ulcers as well as cysteamine –induced duodenal ulcers. The patol churna extract was showed significant anti-ulcer activity ($p<0.05$) against both gastric (reduction in ulcer index) and duodenal ulcers (reduction in total lesion area) when compared with cimetidine (100mg/kg, p.o). [Varsha J *et al.,* 2011]

ANTI-BACTERIAL ACTIVITY

Different extracts of (petroleum ether, chloroform , ethanol &distilled water)*T.cucumerina* (whole plant)was showed anti-bacterial activity against gram negative[*E.coli* (ATCC 25922), *K.pneumoniae* (NCIM 2719*), P.aeruginosa* (ATCC 27853)]& Gram positive bacteria[*S.aureus* (ATCC 25923)].The zone of inhibition(12.50 to 19.61 mm)of antimicrobial activity was more in water & ethanol extracts than in chloroform and petroleum ether extracts.[Devendra N *et al.,* 2009][b]

Hot water(56.25 µg/ml)&cold ethanolic (28.12µg/ml) extracts of *T.cucumerina* showed significant anti-bacterial activity(disc diffusion technique)against Gram positive and Gram negative strains(*Staphylococcus aureus* NCTC 25923*, Streptococcus pyrogens* NCTC 20258, *Escherichia coli* NCTC 25922 and *Pseudomonas aeroginosa* NCTC 20620) .[Arawwawala LD *et al.,* 2011]

DIURETIC ACTIVITY

Different extracts of (petroleum ether, chloroform, methanol) *T.cucumerina* reported to show diuretic activity in male wistar albino rats. The methanol extract (100mg/kg p.o)of *T.cucumerina* showed significant (increased urine volume &urine concentration of Na^+, K^+, &Cl^-)diuretic activity($p<0.01$)when compared with Furosemide(25mg/kg).This report support the *T.cucumerina* use in the traditional medicine as diuretic.[Raama Murthy *et al.,* 2012]

ANTHELMINTIC ACTIVITY

Different extracts of *T.cucumerina* showed potent anthelmintic activity against *Pheretima posthuma* .The methanolic extract showed significant ($p<0.001$) activity than petroleum ether and chloroform extracts when compared with standard (Albendazole). [Raama Murthy *et al.,* 2012]

AERIAL PARTS

ETHNOMEDICAL INFORMATION

Traditionally, decoction of the aerial parts were used in the treatment of diabetes and inflammatory diseases. [Kirana H, Srinivasan B, , 2008]

PHYTOCHEMICAL STUDIES

Aerial parts of *T.cucumerina* contains a galactose-specific lectin and ribosome-inactivating protein (trichoanguin).The bulk of carotenoids made of lutein is present in the concentration of 15.6-18.4 mg/100g FW.[Chow LP *et al.,* 1999, Anuradha P and Bhide SV., 1999, Azeez MA and Morakinyo JA., 2004]

Hot water extract of *T.cucumerina* aerial parts revealed the presence of alkaloids, polyphenols, flavonoids, steroids, saponins and tannins.).[Arawwawala LD *et al.,* 2010][a]

PHARMACOLOGICAL STUDIES

ANTI OXIDANT ACTIVITY

Aerial parts of *T.cucumerina* extract (HWE) were showed total phenolic content (65.8±0.50 mg gallic acid equivalents /g extract) and total flavonoid content(45.4±0.76 mg quercetin equivalents /g extract)).[Arawwawala LD *et al.,* 2010][a]

ACID SECRETION STUDY

Oral administration of T.cucumerina aerial parts (HWE) was investigated for acid secretion in rats .Significant reduction in free acidity (40%) and total acidity in gastric juice (36%), pH of gastric juice increased (4.1 to 6) but no change in volume of gastric juice.).[Arawwawala LD *et al.,* 2010] [a]

GASTRIC MUCUS STUDY

Aerial parts of *T.cucumerina* (HWT by oral) were significantly ($p<0.05$) increased the amount of mucus in gastric mucosa (263.1±4.4µg/stomach)compared with control (189.6±8.8µg/stomach). [Arawwawala LD *et al., 2010*] [a]

ANTI HISTAMINE ACTIVITY

Oral administration of *T.cucumerina* (Hot water extract) aerial parts (750mg/kg) showed potent anti-histamine activity in rats. Significant ($p<0.05$) reduction (27%) in HWE of *T.cucumerina* when compared with standard(chlorpheniramine).[Arawwawala LD *et al.,* 2010][a]

GASTROPROTECTIVE ACTIVITY

Oral administration of aerial parts of (HWE) *T.cucumerina* (750mg/kg)showed gastroprotective activity in indomethacin-induced gastric lesions in rats. Significantly ($p<0.05$) reduced the length (88%)and number of gastric lesions(84%)compared with control. Similar gastroprotective activity (90%) was compared with standard on absolute ethanol induced gastric ulceration model.).[Arawwawala LD *et al.,* 2010] [a]

ANTI DIABETIC ACTIVITY

The hot water extract of *T.cucumerina*(aerial parts) has been noted to improve glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats .The drug possess antidiabetic activity with improvement in oral glucose tolerance and glucose uptake in peripheral tissues.[Arawwawala M *et al.,* 2009]

Hot water extract of *T.cucumerina* (750 mg/kg) aerial parts showed significant ($p<0.05$) anti-diabetic activity in STZ induced diabetic rats. .[Arawwawala LD *et al.,* 2011]

LEAVES

ETHNOMEDICAL INFORMATION

Leaf is deemed alexiteric; astringent, **diuretic** and emetic .In the Konkan, the leaf juice is rubbed over the liver in liver congestion, or over the whole body in remittent fevers. [Linn TK., 2012]

Leaf is **cardiotonic,** anti-pyretic, anti-periodic useful for intestinal worms and its juice rubbed over the liver in remittent fever, skin disease, biliousness, emetic, externally applied over bald patches of alopecia.[Sandeep Kumar S *et al.,* 2013]

PHARMACOGNOSTICAL STUDIES

T.cucumerina leaves are alternate, simple with no stipules. Leaves are scabrid hairy on both surfaces, rounded in outline, 7-14 cm long and broad and 3or 5-lobe, the lobes being broad, rounded or obtuse and the sinuses broad or narrow and rounded. The base is broadly heart-shaped. [Anonymous., 2002]

Transverse section of *T.cucumerina* leaf showed upper epidermis , covering trichomes , mesophyll , palisade cells , spongy parenchyma, lower epidermis , midrib , vascular bundles , collenchyma and stomata.[Sandhya S *et al.,* 2010]
Leaf explants of *T.cucumerina* were cultured [Murashige and Skoog (MS)medium]with growth regulators[(2, 4dichloro phenoxy acetic acid (2, 4-D), alpha-naphthalene acetic acid(NAA), Indole butyric acid (IBA), Indole acetic acid (IAA), cytokinins (kinetin)and Benzyl adenine(BA)].Different concentrations and combinations of BAP+IBA, 2, 4-D+BAP&2, 4-D+Kn produced the highest total combinations 4.9%w/w(2, 4-D(3.0mg^{-1})and cucurbitacin E 2.75 %w/w at third week(Kin (1.0 mg^{-1})[Devendra NK *et al.,* 2012]

PHYTOCHEMICAL STUDIES

T.cucumerina leaves were showed the presence of carbohydrates, alkaloids and saponins.[Sandhya S *et al.,* 2010]

Preliminary phytochemical screening of *T.cucumerina* leaf aqueous extracts revealed the presence of carbohydrates, flavonoids, saponins, flavanol glycoside and triterpenoid saponins. [Sandhya S *et al.,* 2012]

Preliminary phytochemical screening of *T.cucumerina* leaf ethanolic extract revealed the presence of polyphenolics, flavonoids, carotenoids, non-protein thiols, vit C and carbohydrates.[Sandeep Kumar S *et al.,* 2013]

PHARMACOLOGICAL STUDIES

LARVICIDAL EFFECT

The acetone extract of *T.cucumerina* leaves showed moderate larvicidal effects. [Rahuman AA*, Venkatesan* P.*,* 2008]

ANTI-BACTERIAL ACTIVITY

Different leaf extracts of *T.cucumerina* (petroleum ether , chloroform , ethyl acetate and methanol)showed significant antibacterial activity(disc diffusion method)against gram positive bacteria[*Bacillus aereus*(MTCC-1305), *Staphylococcus aureus* (MTCC-96), *Enterobacteria faecalis* (MTCC-5112)and *Streptococcus faecalis*(MTCC-439)]and gram negative bacteria [*Salmonella paratyphi*(MTCC-735), *E-coli*(MTCC—729), *Klebsiella pneumonia*(MTCC-109), *Pseudomonas aeruginosa*(MTCC-647), *Proteus vulgaris*(MTCC-426)and *Serratia marcescens*(MTCC-86).Methanol , ethyl acetate , and chloroform extracts of *T.cucumerina* leaves are used as a potential external antiseptic and antimicrobial

activity due to the presence of phenolic compounds , flavonoids and carotenoids.[Joji Reddy *et al.,* 2010]

HAIR GROWTH PROMOTING ACTIVITY

Aqueous leaf extracts of (150&300 mg/kg) *T.cucumerina* was evaluated for hair growth promoting activity with standard (2%minoxidil) on wistar albino rats. *T.cucumerina* leaf extract produced a very good hair growth promoting activity(hair growth completion , length of hair , percentage of hair follicles and diameter of bald patch and concentration of minerals in the blood)comparable to standard .This study supports the traditional claim.[Sandhya S *et al.,* 2012]

ACUTE TOXICITY AND DETERMINATION OF LD_{50}

Oral administration of ethanolic extract of *T.cucumerina* leaves were studied(OECD guidelines) for the lethal toxicity in rats(1300mg/kg bw) upto 72hr and evaluated for the cytotoxicity, difference between hepatorenal bio-chemical parameters(SGOT , SGPT, ALP, Urea , uric acid , creatinine)and histopathological slides of liver and kidney in rats. *T.cucumerina* leaf extract showed no signs of toxicity, no significant difference ($p>0.05$) between biochemical parameters and no changes in anatomy of liver and kidney. Hence this results suggest that ethanolic extract of *T.cucumerina* may safely use for therapeutical benefits. [Sandeep Kumar S *et al.,* 2013]

STEM:

PHARMACOGNOSTICAL STUDIES

T.cucumerina stems are slender, green, 4- angled, somewhat hairy and faintly disagreeable in odour. [Sandhya S *et al.,* 2010]

FRUITS

ETHNOMEDICAL INFORMATION

Diabetic patients are advised to consume young fruits as it is having low sugar and excellent sources of fibers, vitamins, minerals and proteins.[Sandeep Kumar S *et al.,* ., 2013, Linn TK., 2012] Fruit is regarded as anthelmintic , purgative , vomitive [Saboo SS *et al.,* 2013].

PHARMACOGNOSTICAL STUDIES

T.cucumerina fruits are very slender, long and cylindrical berry, often twisted, greenish-white when immature, dark red when mature. [Sandhya S *et al.,* 2010]

PHYTOCHEMICAL STUDIES

T.cucumerina fruit is rich in Vitamin C and E. The crude protein content is 30.18%.[Yusuf AA *et al.,* 2007]

T.cucumerina is a rich source of nutrition. It is highly constituted with proteins, fat fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids content is 46.8% and 78.0% respectively. [Adebooye OC., 2008]

T.cucumerina fruits contain the predominant mineral elements were potassium(121.60mg 100^{-1}g) and phosphorous (135.0 mg 100^{-1}g).Other elements found in fairly high amounts are Sodium, Magnesium and Zinc.[Ojiako OA, Igwe CU., 2008]

Fruit pulp of *T.cucumerina* contained (morphophytes I&II) dry matter[(10.9 & 9.6 g/100g), ascorbic acid(25.7 and 24.8mg/100g), lycopene (18 & 16.1 mg/100g), Bulk of the carotenoids[made up of lutein(15.6 &18.4 mg/100 g), α-carotene (10.3

&10.7mg/100g), β-carotene (2.4&2.8 mg/100g)in fresh weight .This results possessed the valuable neutraceutical properties of *T.cucumerina* can qualify as viable substitute to the Solanaeceous tomato.[Adebooye OC., 2008]

T.cucumerina fruit is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids. [Sandhya S *et al.,* 2010]

Chemical constituents are cucurbitacin B& E, iso cucurbitacin B, 23, 24-dihydro isocucurbitacin B, 23, 24 dihydro cucurbitacin E, sterols 2 β-sitosterol, stigmasterol, α-carotene and β- carotene.). [Sagar L *et al.,* 2012]

Ethanolic extract of *T.cucumerina* fruit revealed the presence of polyphenols , flavonoids, carotenoids, non-protein thiols , vit C and carbohydrates. [Sandeep Kumar S *et al.,* 2013]

Cucurbitacin compounds are characterized by tetracyclic cucurbitane nucleus (triterpenes)with different oxygen substituents at different positions because of their hydrophobic properties and poor water solubility.[Abdullah A., 2013]

PHARMACOLOGICAL ACTIVITY

ANTI-CANCER ACTIVITY

Cucurbitacin B extracted from fresh fruit of *T.cucumerina* were studied anti-proliferative effect on human cancer cell lines[(two human lung non-small cell LK 87 & QG 95), (two human colon cancer cell HCT15 & HT29), (one renal cancer cell line A498), (one pancreatic cancer cell NOR-P)].The ED_{50} values(50% inhibition) of cucurbitancin on human cell lines(69µg/ml in HCT15 cells up to 231µg/ml in QG95 cells).The inhibition of proliferation of cucurbitacin on human cancer cell lines was dose dependent. [Tanawan K *et al.,* 2009]

Cucurbitacin have been isolated from the cucurbitaceae family plant species. Different cucurbtacin compounds showed anti-tumor proliferation inhibition and induced apoptosis in cancer cell models(*in-vivo& in-vitro*).Roots and fruits of cucurbitaceae plant species are very bitter so it is used as folk medicines(anti-inflammatory and anti-cancer).[Abdullah A., 2013]

CARDIOPROTECTIVE ACTIVITY

Methanolic extract of *T.cucumerina* fruit possessed cardio protective activity on doxorubicin (4mg/kg i.p) induced cardio toxicity in wistar rats. Methanolic extract of *T.cucumerina* significantly reduced the cardiac damage(LDH&CK-MB, reduced ST, QT interval and QRScomplex, increased heart rate, restored blood pressure and left ventricular function).[Sagar L *et al.,* 2012]

ANTIOXIDANT ACTIVITY

Fruit pulp of *T.cucumerina* was identified (morphophyte I&II) and showed total phenolics (46.8%), total flavonoids(78%) and total ferric reducing anti-oxidant power were significantly higher($p<0.05$)than that of morphophyte I. [Adebooye OC., 2008]

ACUTE TOXICITY AND DETERMINATION OF LD_{50}

Oral administration ethanolic extract of *T.cucumerina* fruits were studied(OECD guidelines)the lethal toxicity in rats(1300mg/kgbw)upto 72hr and evaluated the cytotoxicity difference between hepatorenal bio-chemical parameters(SGOT , SGPT ALP, Urea, uric acid, creatinine)and histopathological slides of liver and kidney in rats. *T.cucumerina* fruit extract showed no signs of toxicity, no significant difference($p>0.05$)between biochemical parameters and no changes in anatomy of liver and kidney. Hence this results suggest

that ethanolic extract of *T.cucumerina* may safely use for therapeutical benefits.[Sandeep Kumar S *et al.,* 2013]

ANTI-BACTERIAL ACTIVITY

Different extracts of fruit (petroleum ether , chloroform , ethyl acetate and methanol)of *T.cucumerina* were evaluated for anti-bacterial activity(disc diffusion)against gram positive and gram negative bacterial strains (*Bacillus aureus* MTCC-1305, *Enterobacter faecalis* MTCC-5112, *Salmonella paratyphi* MTCC-735, *Staphylococcus aureus* MTCC-96, *Escherichia coli* MTCC-729, *Proteus vulgaris* MTCC-426, *Klebsiella pneumonia* MTCC-109, *Pseudomonas aeruginosa* MTCC-647 and *Serratia marcescens* MTCC-86).Methanol extract of *T.cucumerina* fruit showed anti-microbial activity when compared with standard antibiotics(tobramycin , gentamicin sulphate , oflaxacin and ciprofloxacin)due to the presence of phenolic compounds , flavonoids , carotenoids and terpenoids.[Joji RL and Beena J., 2013]

FLOWERS

PHARMACOGNOSTICAL STUDIES

T.cucumerina staminate inflorescences are long-peduncled and axillary, with 6-15 flowers. Flowers are unisexual, regular and white in colour with green and hairy calyx. Corolla is tubular in with lobes fringed and hair like outgrowths. The male flowers are many flowered with axillary racemes on 10-30 cm long peduncles. They are with 3 stamens but the female flowers are solitary and sessile with inferior, single celled ovary, long and with hairy stigmas. [Sandhya S *et al.,* 2010]

SEEDS

ETHNOMEDICAL INFORMATION

The seed is said to be cooling .The dried seeds are used for its anthelmintic and anti-diarrhoeal properties .Seeds is used as abortifacient, acrid, aphrodisiac, astringent, bitter, febrifuge, purgative, toxic, trichogenous.Seeds have anti-bacterial, anti-spasmodic and insecticidal properties [Madhava KC *et al.,* 2008, Joji RL *et al., .,* 2010, Linn TK., 2012], anti-diabetic , anti-febrile , stomach disorders.[Saboo SS *et al.,* 2013,]

PHARMACOGNOSTICAL STUDIES

T.cucumerina seeds are half-ellipsoid, somewhat compressed, undulate, hard, rugose, nearly 1cm long, greyish-brown, sculptured, margin undulate and embedded in a soft foetid with red pulp.[Kritikar KR and Basu BD., 2006]

PHYTOCHEMICAL STUDIES

T.cucumerina seed contain cucurbitacin B, cucurbitacin E, isocucurbitacin B, 23, 24- dihydroisocucurbitacin B, 23, 24-dihydrocucurbitacin E, sterols 2 β-sitosterol stigmasterol and low amount of chemical substances like oxalate, phytates, tannins etc also present.[Datta SK., 1987]

The positive effects of *T.cucumerina* are due to the presence of carotenoids, flavonoids, lycopene, phenolics and β-carotene. [Patil AS and Bhole SR., 1993]
A novel isoflavone glucoside, 5, 6, 6′-trimethoxy-3′, 4′-methylenedioxyisoflavone7-O-beta-D-(2″-O-p-coumaroylglucopyranoside)has been characterized from the seeds of *Trichosanthes*.[Yadava RN and Syeda Y., 1994]

Chemical modifications carried out with imidazole side chains of histidine residues with ethoxyformic anhydride on the galactose-specific lectin (SGSL) purified from snake gourd. *Trichosanthes* seeds indicated that the loss of activity upon modification was not due to changes in the overall conformation of the lecithin. [Chanchai S., 1993 and Swamy MJ *et al.,* 1998]

T.cucumerina seeds contains a galactose-specific lectin and ribosome-inactivating protein (trichoanguin).The bulk of carotenoids made of lutein is present in the concentration of 15.6-18.4 mg/100g FW.[Chow LP *et al.,* 1999, Anuradha P and Bhide SV., 1999]

Circular dichroism spectroscopic studies reveal that *T.cucumerina* seed contains beta -sheet(28.4%), beta-returns(10.6%), polyproline type 2 structure(7%) with unordered structure of α-carotene(10.3-10.7mg/100g FW)and β-carotene(2.4-2.8mg/100g FW)[α-helix content is negligible], ascorbic acid(24.8-25.7mg/100g FW), lycopene(16.0&18.1mg/100g FW).[Paadma P, Komath SS., 1999, Kenoth R *et al.,* 2003]

T.cucumerina seed analysis showed high oil content upto 42.5±5%.The presence of common protein bands among the species may be an evidence of evolutionary origin and many protein bands found to be unique in the *T.cucumerina* suggested that there is no genetic relationship with *Lycopersicon* .[Ekam VS., 2003]

PHARMACOLOGICAL ACTIVITY

Chloroform and ethanol extract of *T.cucumerina* seed (200&400 mg/kg p.o) showed anti-inflammatory activity in wistar rats(carrageenan induced paw oedema).Significant activity in ethanol & chloroform extract. This results support to

the traditional medicine of *T.cucumerina* used as anti-inflammatory. [Devendra K *et al.,* 2010]

ROOTS

ETHNOMEDICAL INFORMATION

Two ounces.The bulbous part of the root is considered as a hydragogu and cathartic .In china, peptides in the plant are used as an abortifacient, the roots used for diabetes, skin swellings like boils and furnuncles. Fresh root has anti-convulscent activity and the root sap has a potent purgative action. [Linn TK., 2012]

PHYTOCHEMICAL STUDIES

T.cucumerina roots were characterized a novel iso flavone glucoside, 5, 6, 6'-trimethoxy 3, 4-methylene-dioxy iso flavone 7-0-beta-D-(2'-0 pcoumaroyl glucopyranoside). [Sagar L *et al.,* 2012]

PHARMACOLOGICAL ACTIVITY

ANTI CANCER ACTIVITY

The root and fruit juice extract of *T.cucumerina* were tested cytotoxicity against four human breast cancer cell lines and lung cancer cell lines and one colon cancer cell line. The root extract inhibited more strongly than the fruit juice. [Kongtun S *et al.,* 1999]

Root extract of *T.cucumerina* and bryonolic acid (1), fruit juice of *T.cucumerina*, cucurbitacin B were tested for cytotoxicity against 4 human breast cancer cell lines and two lung cancer lines and one colon cancer cell line. Root extract shown higher IC_{50} value than bryonolic acid against 3 breast cancer and 1 lung cancer cell lines. Fruit juice shown higher IC_{50} value than cucurbitacin B against 4 breast

cancer, 1 lung cancer, 1 colon cancer cell lines extract inhibited SK-LUI more strongly than fruit juice .Bryonolic acid inhibited MDA-MB435 somewhat better than other cells. Fruit juice inhibited colon cancer cell line more strongly than root extract. Cucurbitacin inhibited more strongly than bryonolic acid.Bryonolic acid 1 & 2, cucurbitacin B, dihydro cucurbitacin were isolated from root extract.[Kongton S *et al.,* 2009]

ANTI INFLAMMATORY

Root tubers of *T.cucumerina* (Hot Water Extract) were showed significant anti-inflammatory activity against carrageenan induced mouse's hind paw oedema. [Kolte RM *et al.,* 1997]

CHAPTER -3

AIM & OBJECTIVE

CHAPTER - 3

AIM AND OBJECTIVE

India is well known for its rich traditional system of medicine i.e Ayurveda , Siddha, Unani etc besides a vast resources of ethno medicine. Indian rural households are limited to access health services practice home remedies which have been handed down from generation to generation. The medicines of the plant origin are preferred over animal origin due to abundances. Indian system of medicine insists on the maintenance of health or swastha a distinguishing feature,the prescribed procedure emphasis on drugs along with daily routine including excercise, diet, nutrition besides mental attitude and discipline. This is achieved by using extracts of various plants (the rasayanas). Indian medicinal plants are highly rich sources of constituents that have several therapeutic properties like cardio protective, chemo preventive and other effects. In Ayurveda it is clearly mentioned the any patients can be cured with the help of herbs present in the surroundings (Lele RD, 2001, Upadhaya, 1995). So we have selected a member of the family cucurbitaceae, a dietary plant snake gourd known botanically as *Trichosanthes cucumerina* Linn. The leaves of *Trichosanthes cucumerina* really do not have any match as a cheap, natural and easily available edible plant and useful for the treatment of many diseases. Leaf is used as cardiotonic, hair growth promoting agent and for wound, diabetes etc., Hence research and development of herbal products from this plant is required to be initiated immediately for exploring the unique potential of this crop which would also minimize the menacing wastage especially the leaves.

Cardio vascular disease (CVDs) contributes a leading cause of mortality worldwide India and other developing countries struggling to manage CVD and the

growing burden of obesity, hypertension and diabetes. Current survey suggests that by the year 2020. India will have the largest CVD burden in the world. Hence it is urgent to explore various strategies to combat the increasing risk of CVDs in the Indian subcontinent. Medicinal plants with cardio protective constituents can play a major role in this aspect. Epidemiological studies show an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease (CHD) which is explained in part by the inhibition of low density lipoprotein oxidation and reduced platelet aggregability(Cook NC and Samman S 1996)

The most frequently studied flavonoid, quercetin, has been shown to have biological properties consistent with its sparing effect on the cardiovascular system. Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses), protect low-density lipoprotein from oxidation (prevent atherosclerotic plaque formation), prevent platelet aggregation (antithrombic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects).(Fromica JV and Regelson W 1995)

Our interest in flavonoids arised from the fact that they are present in the leaves of *T. cucumerina*.

The **3R's** ethical principle (**R**eduction, **R**efinement, and **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science. We planned to carry out both *in vivo* and *ex-vivo* study without using mammalian system in this drug discovery process and also to help cut cost and save time.

As the small animals models are emerging to perform *in vivo* testing. We have preferred *D.magna* (water flea) **for transparent myogenic heart** and possible to count heart beat were attracted us to undertake this studies *in vivo* to screen cardio protective study.

AIM:

To study the pharmacognostic , preliminary phytochemical, effect on lactose induced arrhythmia on *D.magna* myogenic heart along with enhancement of *ex-vivo* Porcine skin wound healing model of the leaves of *T.cucumerina* Linn. (Family: Cucurbitaceae).

OBJECTIVE:

The objective of the study was divided into three parts.

Part 1: Phramacognostical study:-

- Collection and authentication of plant
- Macroscopy of the plant
- Microscopy of the leaf
    1. Anatomical study
    2. SEM analysis
    3. Powder microscopy
    4. Microscopic schedules
- Physio-chemical parameters
    1. Ash value
    2. Loss on drying
    3. Extractive value

Part 2: Preliminary Phytochemical Screening:-

- Preparation of ethyl acetate extract of *T.cucumerina* leaves (TCEAE))
- Qualitative analysis of the leaves for the presence of various phyto constituents
- Determination of flavonoid content, total phenolic content, determination trace elements by EDS
- Identification and quantitative determination of common flavonoids by HPTLC

Part 3: Pharmacological study: - (to improve the R&D landscape of cardioprotection and wound and to bridge the lacunae in ethnomedical uses and scientific background)

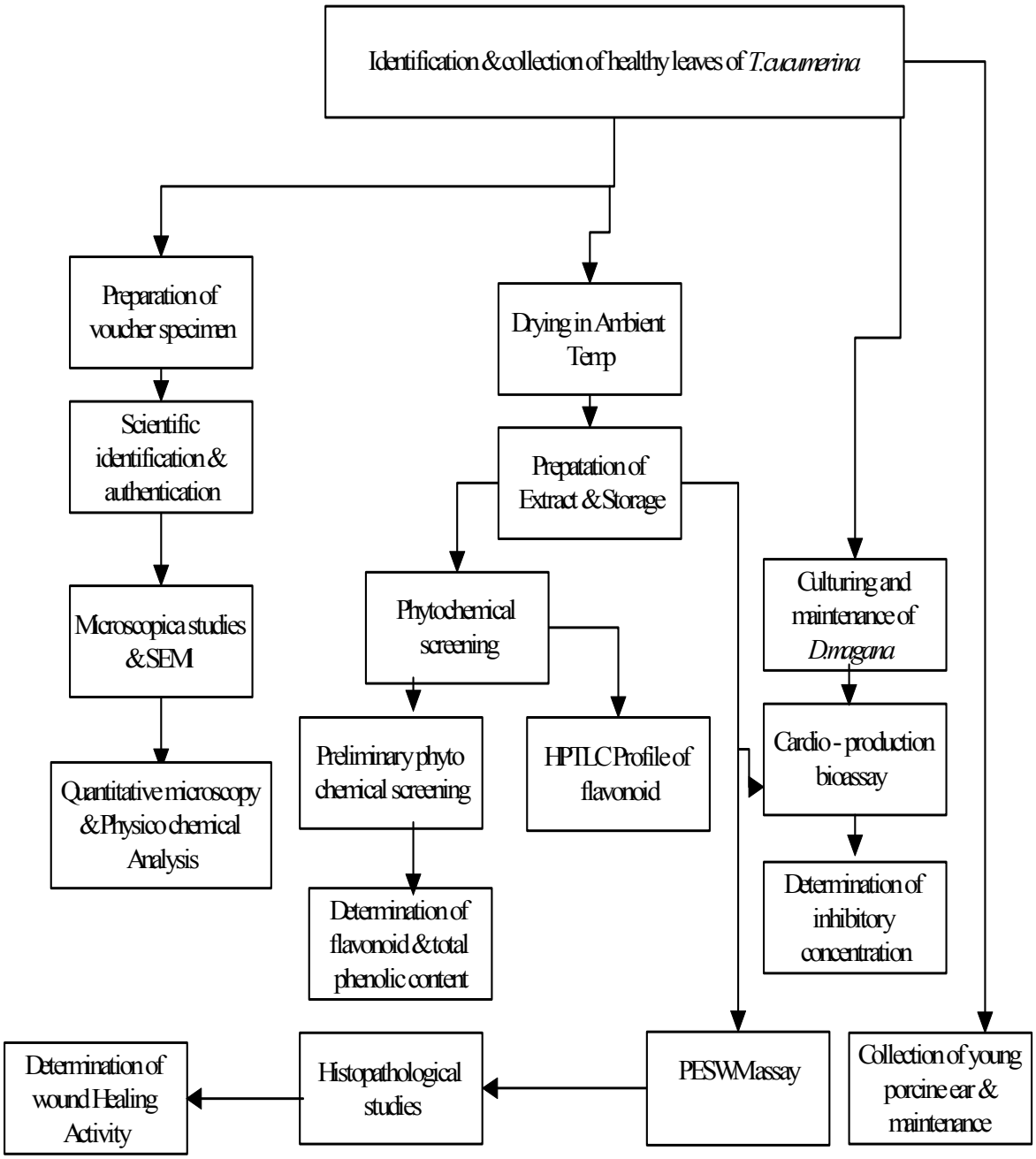
- Collection and authentication of *D.magna*
- Preparation of the Elendt-Bias (M4) medium
- Culture of *D.magna*
- To assess the **acute toxicity** of TCEAE on the *Daphnids*
- **To evaluate the effects of TCEAE on the lactose induced cardiac arrhythmia on the myogenic heart of *D.magna*. as a validation of ethnopharmacological use as cardiotonic**
- To evaluate the effect of TCEAE on the *ex-vivo* Porcine Skin Wound Healing Model (PSWHM) to provide scientific confirmation of the traditional claim in the treatment of wound

CHAPTER-4

MATERIALS AND METHODS

CHAPTER – 4
MATERIALS AND METHODS

RESEARCH DESIGN



4.1. PLANT COLLECTION AND AUTHENTIFICATION

Leaves of the plant *Trichosanthescucumerina*Linn. selected for our study was collected from **Tenkasi, Tirunelveli District**, Tamil Nadu, India during the month of July 2013 and was authenticated by**Dr.Stephen,** Department of Botany, American college, Madurai and**Dr.Sasikala**, Director of Siddha Research centre, Arunbakkam, Chennai.

Leaf drying and pulverizing

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

4.2. PHARMACOGNOSTIC STUDIES

Morphological and micro morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work.

4.2.1. Morphological studies of *Trichosanthescucumerina*Linn.

Aerial part, leaf and petiole, flower and fruits were studied individually for its morphological characters by organoleptic test.

4.2.2. Microscopical studies on the leaf of *T.cucumerina*

Collection of specimen

Care was taken to select healthy plants and for normal organs. Leaf, Petiole specimens were collected from a healthy plant by making a cut with petioles.The materials were cut into pieces and immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml).

Dehydration

After 24 hours of fixing , the specimens weare dehydrated with graded series of ethyl alcohol and tertiary-butyl alcohol as per the schedule given by Sass, 1940. The specimen is kept is in each grade of the fluid for about 6 hrs.Every time the fluid is decanted and immediately the specimen were flooded with next grade of fluid.

Infiltration with paraffin wax

After dehydration, the shavings of parafin wax were added to the vial containing the plant material with pure TBA. The paraffin shavings are added every 30mts at about 40-45ºC four or five times. Then the vials were filled with wax without damaging the tissues. The vial filled with wax is kept open in warm condition to evaporate all TBA, leaving the specimen in pure molten wax. The specimen filled with pure molten wax for 2 or 3 times by decanting the old wax every time.

Casting to mold

A boat made out of chart board, by folding the margin, is used to prepare a mold of wax containing specimens. The paraffin along with the leaf and petiole specimen was poured into the boat. With the help of heated needles, the specimen were arranged in parallel rows with enough space in between the specimens. The block was then immersed in chilled water and allowed to cool for few hours.

Sectioning:

The paraffin embedded specimens were sectioned with the help of microtome**.** The thickness of the sections was 10-12µm. Dewaxing of the sections was by customary procedure. The sections were stained with **Toluidine blue** as per the method published by O'Brien *et al* (1964). Since toluidine blue is a poly chromatic strain, the straining results were remarkably good and some **cytochemical reactions** were also obtained. The dye rendered pink colour to the **cellulose** walls, blue to the lignified cells, dark green to **suberin**, violet to the **mucilage**, blue to the **protein** bodies etc. Whereever necessary sections were also stained with **safranin** and **fast-green** and potassium iodide (for starch).

For studying the stomatal morphology, venation patttern and trichome distribution, **paradermal sections** (sections taken parallel to the surface of leaf as well as **clearing** of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken.with Nikon labphot 2 Microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light were employed. Since these structures have birefringent property, under polarized light they appear

bright against dark background. Magnifigations of the figures are indicated by the scalebars. (Johansen DA, 1940, Purvis MJ *et.al*., 1966).

4.2.3. POWDER MICROSCOPY:

Maceration technique

Maceration is the process of separation of individual cells by selectively dissolving the pectic middle lamella between the cells. The middle lamella binds the cells with each other forming different tissues. The middle lamella is dissolved by employing a chemical that dissolves the lamella to free the cells to obtain their three dimensional view.

Maceration fluid

Jaffrey's maceration fluid is one that is commonly used for maceration (Johnsen, 1940). The fluid consists of equal volumes of 5% chromic acid and 5% nitric acid. The plant material is cut into small pieces and immersed in the maceration fluid. The fluid with the materials is kept at 55ºC for 3-5 hrs. Then the material is washed thoroughly with water and placed on a glass slide in a drop of safranin (0.5%) for 15-20 min. The stain is drained carefully and mounted with a drop of dilute glycerin. The cells are spread well with a needle and the material is covered with cover slip. The slide so prepared is examined under the microscope to study different components of the macerate.

4.2.4. MICROSCOPIC SCHEDULES (Wallis TE, 1953, Wallis TE, 1965, Iyengar MA, 1994, Anonymous, 2001)

The vein islet number, vein terminal number, stomatal number and stomatal index were determined on fresh leaves using standard procedures.

A. Vein islet number and Vein termination number

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islets per sq.mm. Area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq., mm. area of the photosynthetic tissue.

B. Determination of Vein Islet Number and Vein Termination Number

Small square portion from the lamina region of the leaf was cleared in chloral hydrate, stained and mounted on a slide. A camera Lucida is set up and by means of a stage micrometer the paper is divided into squares of $1mm^2$ using a 16mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are raced in four continuous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides. Ten readings for vein islet and vein termination number were recorded.

C. Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I, Stomatal index = S/S+E x 100

Where S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

D. Determination of Stomatal Index

The procedure adopted in the determinations of stomatal number was observed under high power (45 X).The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula.

4.2.5. MICROSCOPICAL STUDY OF*T.cucumerina* LEAF USING SCANNING ELECTRON MICROSCOPE

Scanning Electron Microscope (SEM)

Movement of beam of foccussed electrons across an object forms a 3D image on a cathode - ray tube in a Scanning Eectron Microscope and it reads both the electrons scattered by the object and the secondary electrons produced by it. The electromagnetic lenses are used in SEM and focussing is done by the current. On photographic plate of screen the image is projected which gives comprehensive, quasi 3-D representation of the objects gives the ultra structure of plant cells. In addition , shows the unsuspected details and any undescribed characters. In other words the micrograph from SEM, shows the best possible structural details of the specimens. (Robards, 1970)

Usage

SEM info was handled as conventional character (or) character complexes as “pure” information without being broken down (or) interpreted as individual character using computer processing. The SEM information can be used some what at the superficial level just described to assist in solving taxonomic problem by confirming, changing (or) other grounds. It is also used often as diagnostic feature to avoid misleading by over simplified descriptions and one may find new kinds of

microstructures not previously recognised and apprently simple structures may be extremely complex. Remarkably, poor conventional descriptions enabling taxonomic process of reducing a complex pattern to a few simple characters (Heywood VH, 1971). SEM plays a vital role when a specimen need to be satisfactorily defined in terms of characters. For most biological materials, maximum information is obtained by employing light and electron microscopy jointly and an attempt was made by applying SEM to the leaf of *T.cucumerina*, to pinpoint the positions of specific characters with in the cell, which can be easily seen in final image.

SEM sample preparation

Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small sample were mounted with I sq. cm glass slide And kept in carbon adhesive sheet.. Samples were coated with gold to a thickness of 100 AO using hitachi vacuum evaporator. Coated sample were analysed in a Hitachi Scanning electron Microscope 3000 H model.

4.2.6. PHYSICOCHEMICAL PARAMETERS:(Anonymous, 1996, 1998, 2001)

Determination of Ash Values

Ash Value

The ash values were determined by using air dried powder of the leaf as per the official method.

Total ash

Two grams of the air dried leaf powder was accurately weighed in a silica crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding

450°c, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

Water soluble Ash

The ash obtained from the total ash procedure was boiled with 25ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minutes at a temperature not exceeding 450°c. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

Acid insoluble ash

The ash obtained from the total ash was boiled for five minutes with 25ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

Determination of Loss on Drying

For the determination of loss on drying, the method described by Wallis was followed.One gram of the powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP'96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105°C for 1 hour. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Extractive Values (Individual Solvent)

❁Petroleum Ether Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

❁Ethyl Acetate Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of ethyl acetate in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethyl acetate. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the ethyl acetate soluble extractive value was calculated with reference to the air dried powder.

❁Ethanol Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

❁Water Soluble Extractive Value

Five gram of the coarsely powder was macerated separately with 100ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

4.3. PHYTOCHEMICAL STUDIES

[Anoymous, 1998, Chaudhri RD, 1999, Kokate CK, 2005, Agarwal, 2007, HorboneJB, 1973].

4.3.1 PRELIMINARY PHYTOCHEMICAL SCREENING

PREPARATION OF EXTRACT

The leaf powder was sieved in a No.60 sieve and refluxed with ethyl acetate for 4hrs and filtered. The filtrate evaporated under vacuum (Rotavapor RII, Buchi). The pale green residue obtained (TCEAE) was stored in the refrigerator until further use.

TEST FOR ALKALOIDS

Various procedures to liberate alkaloids

- Powdered drug was mixed thoroughly with 1ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.

- Powdered drug was mixed thoroughly with 1ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was ground in a mortar for about 1 minute with 2ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic Aluminium oxide. The mixture was then loosely packed into a glass column and 10ml chloroform was added, eluted, dried and methanol was added.
- Powdered drug was shaken for 15 minutes with 15ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20ml filtrate; 1ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10ml diethyl ether. The ether was dried over anhydrous sodium sulphate, filtered and evaporated to dryness and the resulting residue was dissolved in methanol.
- Powdered drug was mixed with one gram of calcium hydroxide and 5ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water bath. It was then filtered and the alcohol was evaporated. To that dilute sulphuric acid was added.

The above made extracts were tested with various alkaloid reagents as follows.

1. Mayer's reagent

2. Dragendorff's reagent

3. Hager's reagent

4. Wagner's reagent

Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform was treated with 1ml of hydrochloric acid in a porcelain dish and 0.1g of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapors of dilute ammonia solution.

TEST FOR CARBOHYDRATES

Molisch's test

- The aqueous extract of the powdered material was treated with alcoholic solution of α - naphthol in the presence of sulphuric acid**.**

Fehling's test

- The aqueous extract of the powdered material was treated with Fehling's I and II solution and heated on a boiling water bath.

Benedict's test

- The aqueous extract of the powdered drug was treated with Benedict's reagent and heated over a water bath.

TEST FOR GLYCOSIDES

General test

- **Test A**

200 mg of the powdered drug was extracted with 5ml of dilute sulphuric acid by warming on a water bath, filtered and neutralized with 5% sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

❖ **Test B**

200 mg of the powdered drug was extracted with 5ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1ml of Fehling's solution I and II were added, until it becomes alkaline and heated on a water bath for 2 minutes.

Anthraquinones

❖ **Borntrager's test**

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

❖ **Modified Borntrager's test**

About 0.1gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

Test for cyanogenetic glycosides

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

Test for cardiac glycosides

- **Keller Killiani Test**

About 1gram of the powdered leaf was boiled with 10ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3ml of glacial acetic acid containing a trace of ferric chloride. To this 3ml of concentrated sulphuric acid was added along the sides of the test tube carefully.

- **Raymond Test**

To the alcoholic extract of the leaf, hot methanolic alkali was added.

- **Legal's Test**

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro pruside solution were added.

Coumarin glycosides

A small amount of powdered leaf was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

TEST FOR PHYTOSTEROLS

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

❁ **Salkowski Test**

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

❁ **Libermann – Burchard's Test**

To the chloroform solution few drops of acetic anhydride was added and mixed well. 1ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

Test for Saponins

About 0.5gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5ml of the filtrate was then diluted with water and shaken vigorously**.**

Determination of Foaming Index

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90^{o}C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V_1).

Ten Stoppard test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3ml up to 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Then the tubes were Stoppard and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.

TEST FOR TANNINS

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

- **Gold beater's skin Test**

2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

TEST FOR PROTEINS AND FREE AMINOACIDS

- **Millon's test**

The acidulous alcoholic extract of the powdered leaf was heated with Millon's reagent.

- **Biuret test**

To the alcoholic extract of the powdered leaf 1ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

- **Ninhydrin Test**

To the extract of the powdered drug, ninhydrin solution was added, and boiled.

TEST FOR MUCILAGE

To the aqueous extract of the powdered leaf, ruthenium red solution was added.

TEST FOR FLAVONOIDS

- **Shinoda Test**

A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.

- **Alkaline reagent test**

To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

- **Zinc Hydrochloride Test**

To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added.

TEST FOR TERPENOIDS

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and thionyl chloride were added.

TEST FOR VOLATILE OIL

About 100gram of fresh leaves, were taken in a volatile oil Clevenger apparatus and subjected to hydro distillation for four hours.

TEST FOR FIXED OIL

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105^{o}C for 10 minutes.

4.3.2. FLUORESCENCE ANALYSIS

Powdered leaf material of*T.cucumerina* was subjected to analysis under UV light after treatment with various chemical and organic reagents like Ethanol, Ethyl acetate, Chloroform, Water, 50% sulphuric acid, 10% sodium hydroxide, 50% nitric acid and dried leaf powder (Horbone JB, 1973).

4.3.3. ESTIMATION OF FLAVONOID CONTENT

[Chang CC *et al*.,2002, Mabry TG *et al*., 1970 and Siddiquie MA *et al.,* 2010].

The flavonoid content of plant extract was estimated by aluminium chloride method. In this method, aluminium chloride complexes with flavonoids of C3-C5 hydroxyl group and to produce intense colour in acidic medium. The intensity of the colour is proportional to the amount of flavonoids and can be estimated as quercetin equivalent at wavelength of 415nm.

Materials required

1. Ethyl acetate extract of the leaves of *Trichosanthescucumerina*(TCEAE)
2. 10%w/v aluminium chloride
3. 1M Potassium acetate
4. 95%v/v ethanol

Procedure

0.5ml of TCEAE (1mg/ml) was transferred to a test tube. To this solution, 0.1ml of aluminium chloride, 0.1ml of potassium acetate, 1.5ml ethanol and made to 5ml with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The absorbance was measured at 415nm. The calibration curve was generated using quercetin as a standard at different concentrations (5-50µg/ml).

The reaction mixture without aluminium chloride was used as a blank. The flavonoids content in TCEAE was expressed as mg of quercetin equivalent per gram of extract.

4.3.4. ESTIMATION OF TOTAL PHENOLIC CONTENT (Singleton VL *et al*., 1979,Gouthamchandra K *et al.,* 2010)

Principle

The total phenolic content of the TCEAE was determined by FolinCio-calteau reagent. This reagent consists of phosphotungstate and phosphomolybdate mixture which is reduced to mixture of blue molybdenum and tungsten oxides while phenolic content of the extract was oxidized. The intensity of colour is proportional to the amount of phenolic content of the extract and which was measured at 765nm. The total phenolic content in TCEAE was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract.

Materials

1. Ethyl acetate extract of leaves of *Trichosanthescucumerina*(TCEAE)
2. 10%w/v sodium carbonate solution
3. Gallic acid
4. Folin-Ciocalcateau reagent

Procedure

0.5ml and 1ml of TCEAE was transferred into separate test tube. To this solution, FCR 0.5ml and 1ml of sodium carbonate were added and final volume made upto 10ml with distilled water. The mixture was allowed to stand for 1hr with intermittent shaking. The absorbance was measured at 765nm. A calibration curve was generated using gallic acid as a standard at different concentrations (2, 4, 6, 8, 10µg/ml). The reaction mixture without sample was used as a blank. The total

phenolic content inTCEAE was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

4.3.5 DETERMINATION OF TRACE ELEMENTS IN LEAF OF *T.cucumerina* BYENERGY DISPERSIVE X-RAY SPECTROMETER (EDS)

The SEM allows the observation of materials in macro and submicron ranges. SEM is capable of generating 3-D images for analysis of topographic features . When SEM is used along with EDS the analyst can perform an elemental analysis on specimens of microscopic sections or contaminants that may be present.

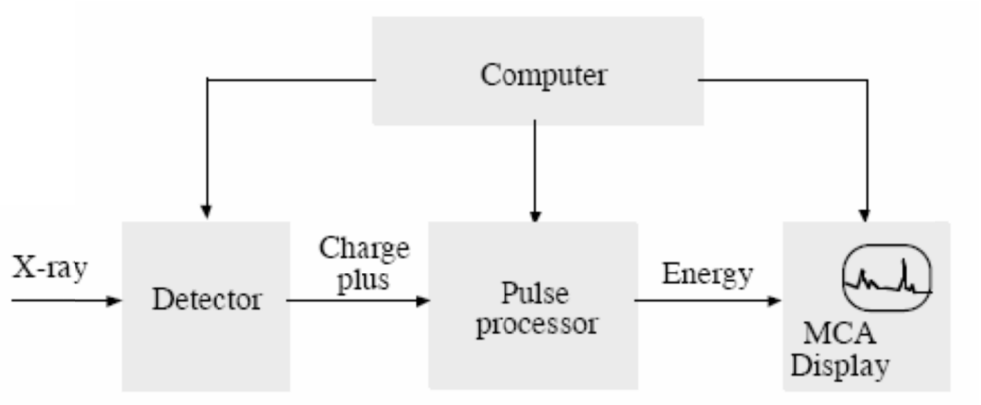
EDS analytical capabilities

Backscattered electron images in the SEM display compositional contrast that results from different atomic number elements and their distribution. EDS is used to find particular elements are and their Atomic % . The Y-axis shows the counts (number of X-rays received and processed by the detector) and the X-axis shows the energy level of those counts[Bob Hofner].

By Viewing 3-D images of specimens solves some of the problem in an analysis and it is also necessary to detect different elements associated with the specimen. This is accomplished by using the “built-in” spectrometer called an Energy Dispersive X-ray Spectrometer.

EDS system comprises of 3 basic components

1. An X-ray Detector - detects and converts X-ray into electronic signals.
2. A Pulse Processor - measures the electronic signals to find out energy of each X-ray detected; and
3. A Multiple Channel Analyser - interprets and displays analytical data.



EDS is an analytical technique in which the specimen emits X-rays due to the bombardment of electron beam on it which is used to identify the elemental composition of the specimen due to the ejection of electrons from the atoms on the specimen surface. To explain further, when the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimens surface. A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted to balance the energy difference between the two electrons. The EDS X-ray detector measures the number of emitted X-rays emitted versus their energy.

The energy of the X-ray is characteristic of the element from which the X-ray was emitted. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for the determinations of the elements.

4.3.6HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY OF TCEAE:

High performance thin layer chromatography (HPTLC) is a modern adaptation of TLC with improved versatility, separation efficiency and detection limits. HPTLC is a useful tool for identification of plant extract because each plant species produces a distinct chromatogram, with unique marker compounds used for the plant identification. It is used as a quality control tool since comparison of chromatograms

of different lots can demonstrate the similarities and differences between the test samples and their standard chemical markers. HPTLC is a reliable method for quantification of nanogram level even when present in complex formation. HPTLC fingerprint analysis is used for rapid identity check, for monitoring purity of drugs, for detection of adulterants, for determining whether a material is derived from a defined botanical species and also to know whether the constituents are clearly characterized (Wagner H *et al.,* 1996)

4.4. PHARMACOLOGICAL STUDIES

Introduction:

Number of therapies available for hypertension including β-blockers, α-blockers, diuretics, ACE inhibitors, calcium channel blockers.Each category work differently among them. All of them having numerous side effect like β-blockers (bradycardia, depression, dizziness, confusion, fatigue), α-blockers (orthostatic hypotension, dizziness, fainting, tachycardia, arrhythmia, drowsiness), diuretics (hypokalemia, hyperuricemia, hyperglycemia, anorexia), ACE inhibitors (dizziness, fainting, light headness, insomnia, dry cough, fatigue), calcium channel blockers (bradycardia, dizziness, fainting, flushing) (Goodman &Gilman, 2011).

Many plants proven as a cardio tonic due to its antioxidant constituents. Plants like *Terminaliaarjuna*, *Allium sativum*(Garlic), *Allium cepa* (Onion), *Asparegusracemosus*, *Cassia fistula*, *Curcuma longa* (Turmeric), *Embilicaofficinalis* (Amla), *Ocimumsantum* (Tulsi), *Phyllanthusamarus*, *Vitisvinifera* (Grapes), *Withaniasomnifera* (Aswaganda), *Zingiberofficinalis*(Zinger) shows cardio tonic activity. Antioxidant and free radical (Hydroxyl radicals, Super oxide anion, Thiyl radicals, Total ROS, Peroxyl radicals, Singlet oxygen) scavenging property mainly responsible for cardio protective activity of medicinal plants (Tilak Jain JA,

Devasagayam TPA, 2006). So there is need for new lead molecule of cardio active drug with lesser side effects.

Principle:

The principles of determination of cardio protective agent on *D.magna* were well enumerated by Bekker, Krijgsman and many scientists at the turn of the century. The discovery of cardio protective drugs made by using *D.magna* was very sensitive, accurate and time bound. Mass screening of cardio active drugs can be done by using *D.magna* assay.

Advantage of *D. magna*screening :(Navarro AV *et al*., 2003, Schleidt S *et al*., 2009,Kass B *et al*., 2009)

1. Genomic sequence of *D.magna*shares most with humans.
2. Its myogenic heart, while most arthropods hearts are neurogenic.
3. Transparent carapace allows easy observation of heart. So apply optical methods to visualize physiological function and to measure several different parameters simultaneously.
4. Non-invasive method.
5. Drugs are directly added to the water in which they swim.
6. In concern of animal welfare, no need of ethical clearance.
7. *D. magna* has similar ANS in compared to humans.
8. Economically feasible. Easy to culture in lab.
9. Less time consuming
10. More sensitive, accurate results.

CULTURE OF *D.magna*:

D.magna obtained from the local aquarium in Madurai, Tamilnadu. It was identified and authenticated by Prof (Major) P.Chandrasekaran, Principal, ManonmaniamSundaranar University Constituent Model College, Vilathikulam, Nagalapuram 628 904, ThoothukudiDt, Tamil Nadu. (Formerly Faculty of PG and Research, Dept of Zoology and Biotechnology, Vivekananda College, Thiruvedakam West 625 217, Madurai, Tamilnadu.*D.magna*was cultured byusing Elendt- Bias (M4) medium and maintained photoperiod ±12hr. spirulina used as a feed in spring water. Aerated for 48hr to obtain O_2 concentration not less than 4mg/ml. experiment was carried out at 20°C±2°C and away from the sunlight (Elendt B, 1990).

4.4.1. ASSESMENT OF ACUTE TOXICITY OF TCEAE BY USING *Daphnia magna*: (Bucher JR, 2002, Adema DMM, 1978)

Toxicology through intensive studies has traditionally focused on the effect of chemicals on living organisms which was done by one chemical at a time. Such approach shows the mode of action of many chemicals and provides a detailed mechanistic understanding of the molecular targets of toxicity for some as the cost of this approach is high. Toxicology studies rely on the utility of vertebrate animals which is an expensive undertaking in both time and cost with debatable predictive power in case of safety aspects for human.

24 hr old *Daphnids* selected for this study. Since neonates may be more sensitive than elder one. Moreover more specificity, simplicity and do not reproduce.

1. *Daphnids* in spring water culture transferred to depression cavity (n=20). No food feed throughout the study.
2. Administrate different concentrations of test drug (1, 2, 3, 4, 5, 6 mg/L of TCEAE) temperature 20°C±2°C maintained.

3. Observe the mortality rate and immobility after 24 hr.
4. LC_{50} was calculated by using probit analysis method.

4.4.2. EVALUATION OF EFFECTTCEAE ON THE ARRHYTHMIC HEART OF *D.magna*: (Campbell AK *et al*, 2004, Navarro *et al*, 2003)

1. Place the *Daphnia* in the depression cavity along with drops of water using glass tube with rubber teats.
2. Divided *Daphnia* into six groups (n=10). Control, lactose induced, test drug treated(20, 40, 60, 80µg/ml), standard drug (Metoprolol) treated (20, 25µg/ml) on lactose induced heart of *D.magna*.
3. Heart beat and rhythm were observed under low power microscope with CCTV and photomicrograph.
4. Results were tabulated**.**

4.4.3 EFFECT OF TCEAE LEAVES ON ex-vivo PORCINE SKIN WOUND HEALING MODEL

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury an inflammatory response occurs and the cells below the dermis begins to increased collagen production. Later the epithelial tissue is regenerated.

The objective of screening of drugs for healing activity based on various measures on healing (Nayak S 2006).

Wound healing evaluated by ex-vivo porcine skin wound healing model (PSWHM). Architecture of pig skin is closer to human (Bollen Peter JA et al, 1999, Laber et al,

2002). Porcine model is an excellent tool for the evaluation of therapeutic agent meant for wound healing (Sullivan TP et al, 2001).

Porcine (6 months old) ears were obtained from a local slaughter house were washed and disinfected with PBS and 70% ethanol respectively. Circular porcine skin (6mm diameter) taken by using circular biopsy punch. Subsequently on the excised skin small circular wound (3mm diameter) made by using circular biopsy punch. Removing epidermis and upper dermis from the centre for making the wound under sterile conditions. The PSWHMs were divided into five groups (n=6). Control, 1%, 2%, 3% test drug (TCEAE) Ointment, Standard drug (Mupirocin) 2% Ointment applied on the wound and immersed in PBS. Incubate the PSWHM with 5% CO2 at 37°C for 2 days. Histopathological evaluation done after staining with hematoxylin / Eosin. The migration was normalized with the PBS group and expressed as mean % ± SE. statistical analysis was performed using one way analysis of variance (ANOVA). P value 0.01 was considered to be statistically significant (Khamule R et al, 2012).

CHAPTER-5

RESULTS

CHAPTER - 5

RESULTS

5.1 PHARMACOGNOSY

5.1.1 Morphological features of *T.cucumerina* Linn.[Kiritikar Basu.,1987]

Leaves : (Plate -2,3)

5-8-12.5 cm long of various shapes lobed a little broader than long,orbicular reniform or broadly ovate,distantly denticulate,more or less deeply 5(rarely 3-7)lobed,the lobes broad, acute,glabrous or nearly show above,less pubescent or when old some times scabrid beneath,base deeply cordate,the sinus often subrectangular, petioles 2.5-7.5cm long,striate,pubescent.

Stems:

3.6-4.5 m long,slender,furrowed,slightly hairy or subglabrous,tendrils 2-3 fid.

Flowers:

Male flowers:

Monaceous male flowers in axillary racemes,with sometimes a solitary male flower from the same axil as the raceme;peduncles of the racemes 5-15 cm long,slender,striate,bearing 8-15 flowers near the apex;pedicels puberulous,8-20 mm long,bracts 0.Calyx-tube dilated at the apex,2-2.5 cm long,about 3mm,wide at the mouth,teeth short,acutely triangular.Petals white,10 mm long,lanceolate-oblong,laciniate at the apex.

Female flowers:

Axillary,solitary or occasionally a female flower in the same axil as the male peduncle;peduncles of female flowers 3-16 mm long.

Plate 1

Habit and Habitat of *T.cucumerina*

Plate 2

Various sizes of leaves dorsal and ventral side

Fig – 1

Diagram of *Trichosanthes cucumerina*

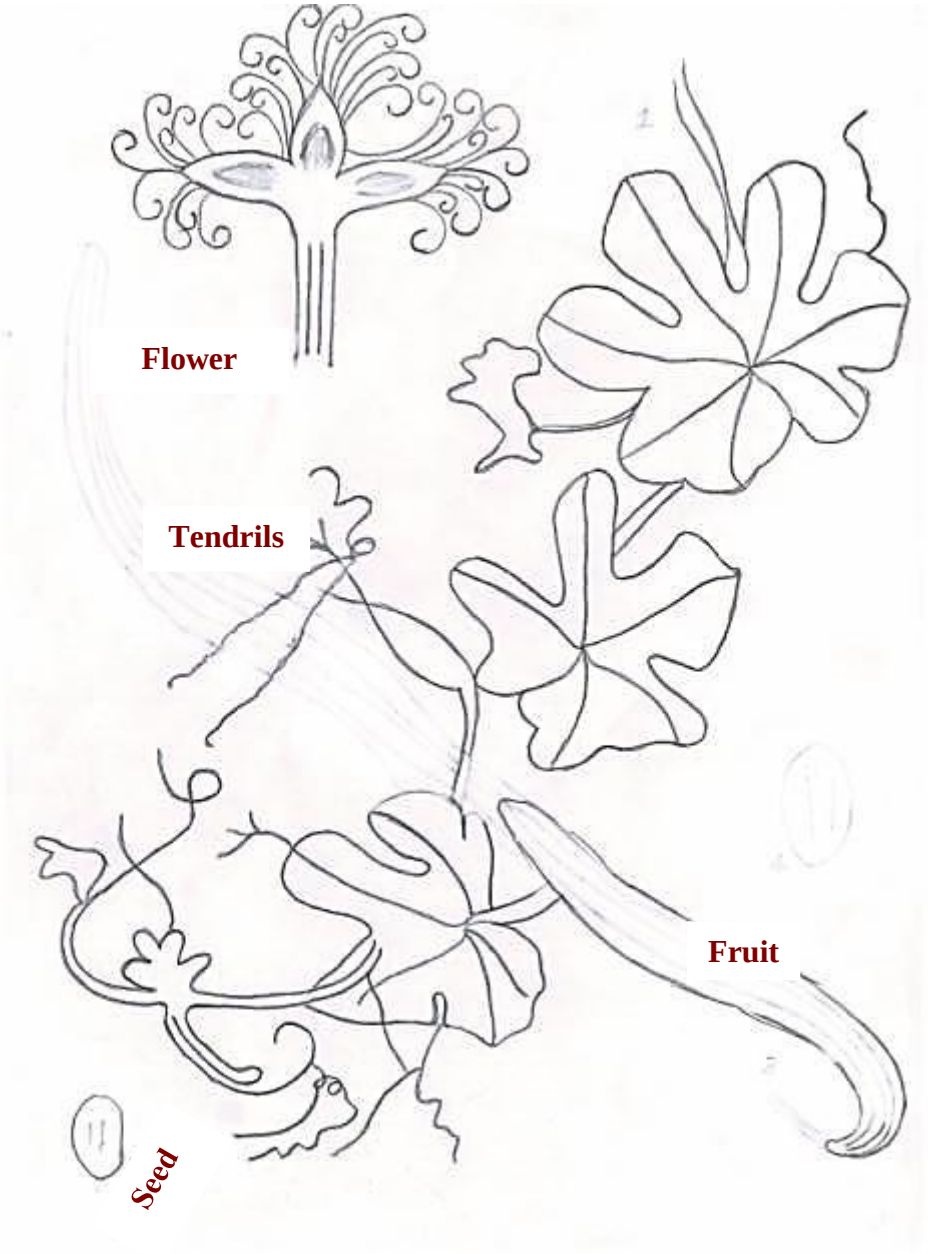


Plate 3

Branch of *T.Cucumerina* with tendrils and fruits showing leaf arrangement

Plate 4

Flower of *T.cucumerina*

Male Flower

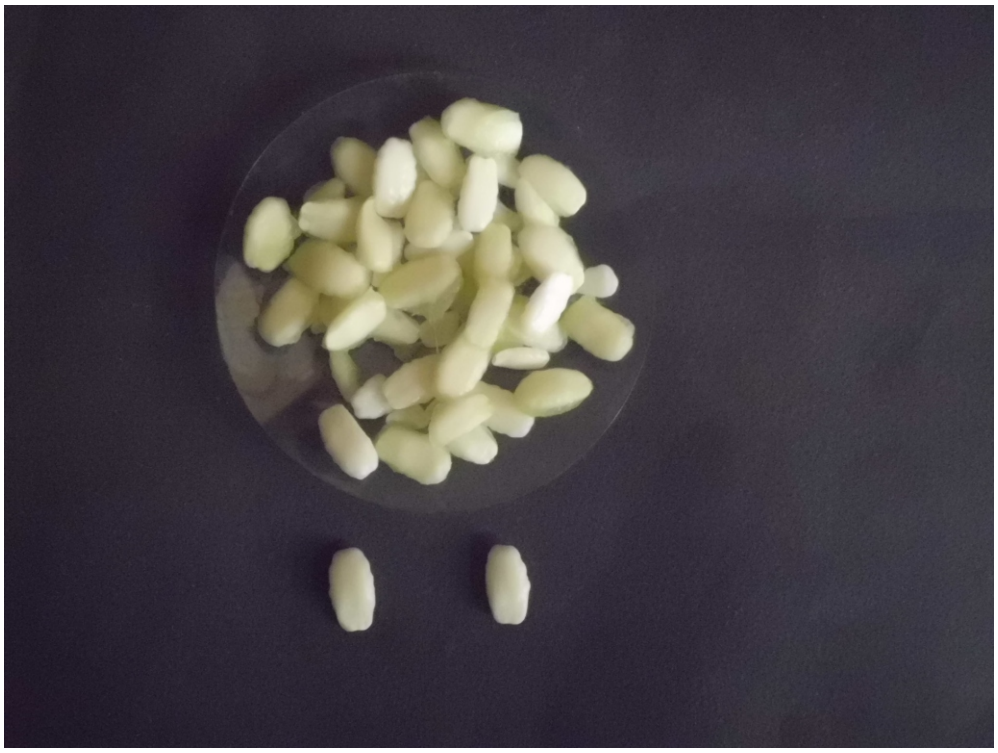
Female Flower

Plate 5

Fruit of *T.cucumerina*

Plate 6

Seed of *T.cucumerina*



Fruit:

2.5-7.5 cm long ,ovoid-fusiform,tapering at both ends and with a long sharp beak,green and striped with white when immature,scarlet when ripe,pericarp thin.

Seeds:

Semi-ellipsoid,compressed,regulose,surrounded with red pulp.

5.1.2 MICROSCOPY OF LEAF:

The leaf is dorsiventral with prominent midrib. Lamina is differentiated..

LEAF MIDRIB (Plate7-9A, Fig-3)

In transectional view

Shape : Plano convex

Adaxial side : raised round hump composed of 3 or 4 layers of Collenchyma cells

Abaxial side : Semi circular convex and hypodermal region Composed of 1 or 2 layers of collenchyma cells.

Epidermis :

Adaxial epidermis: (Plate 2)

Cells are larger in size when compare to the abaxial side and cell walls are wavy. Apostomatic. Some cells are drawn into blunt conical papillae.

Abaxial epidermis: (Plate 9)

Smaller in size and walls are wavy Stomata are ranunculaceous.

Plate - 7

T.S. of Leaf of *T.cucumerina* through the Midrib

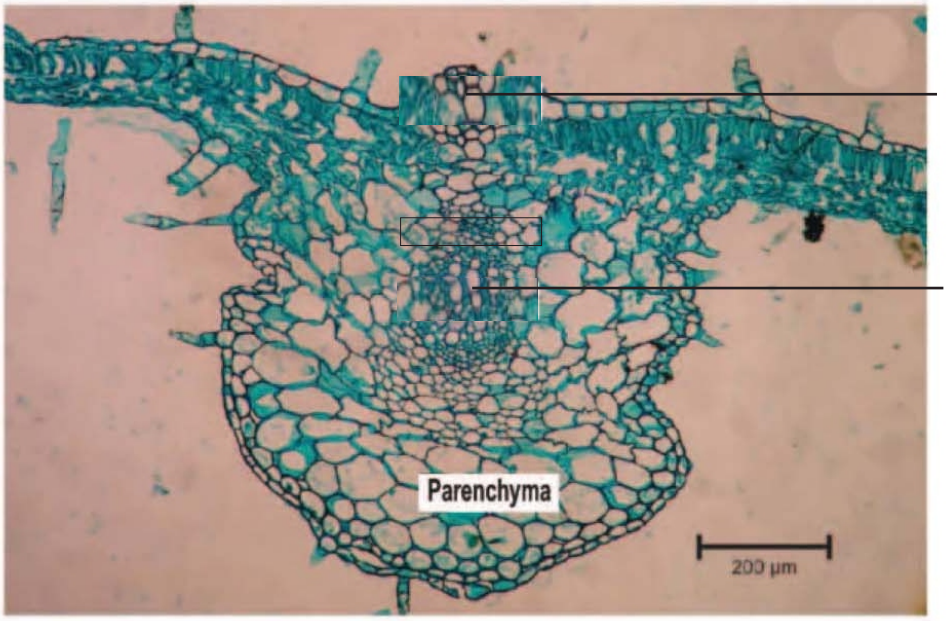


Plate - 8

Upper epidermis (surface view)

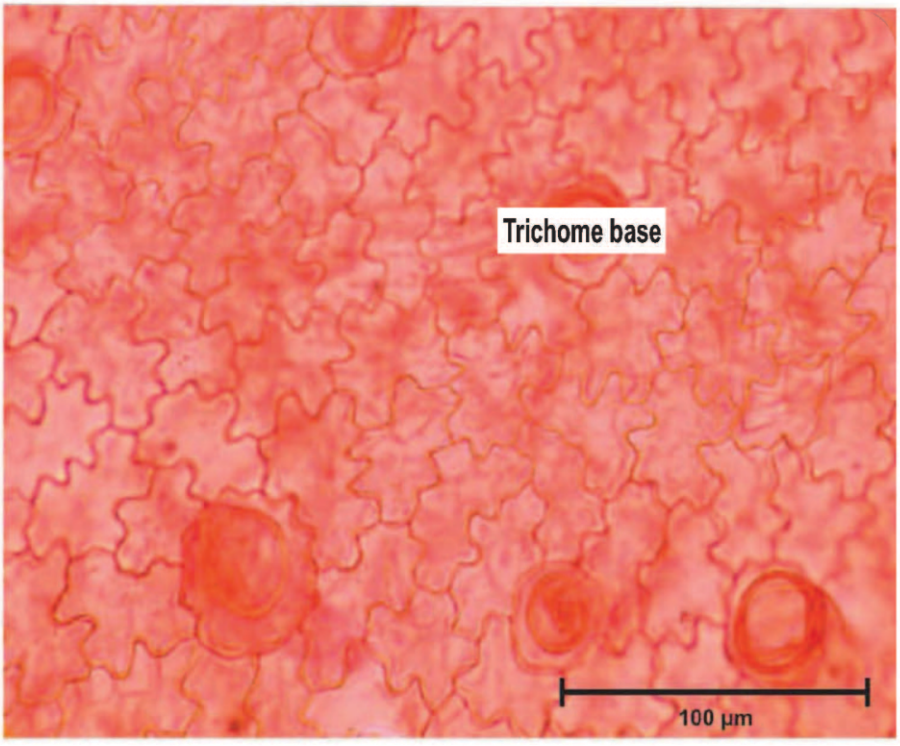


FIG - 3

T.S. of Leaf of *T.cucumerina* through Midrib Ground Plan

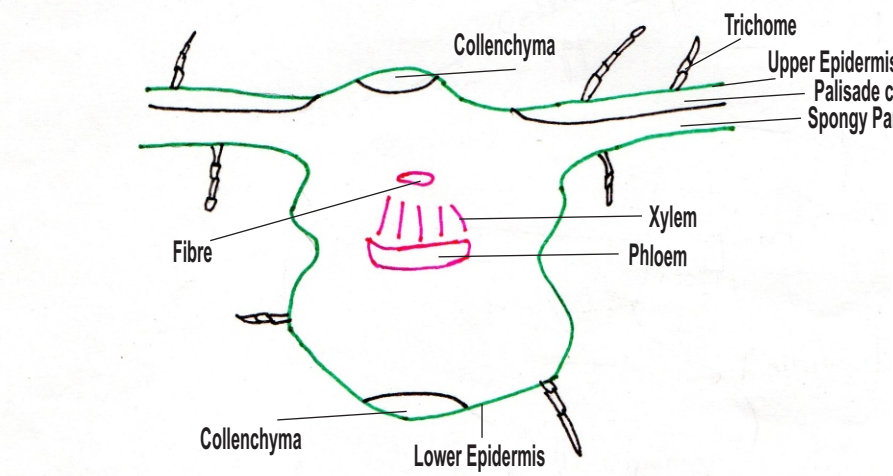


Diagram of T.S of leaf of *T.cucumerina* through Midrib

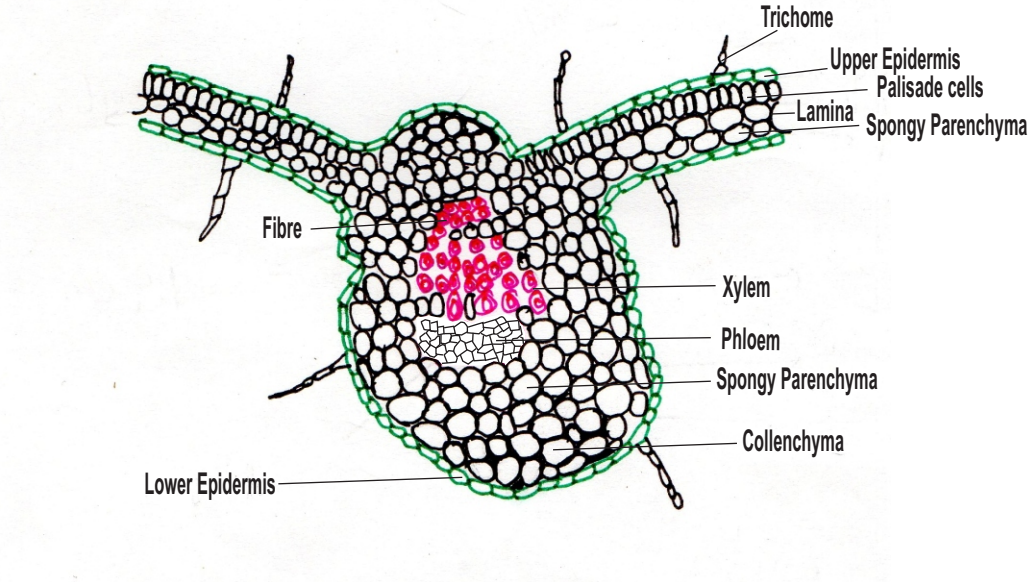


Plate - 8 A
Upper Epidermis - A portion Enlarged

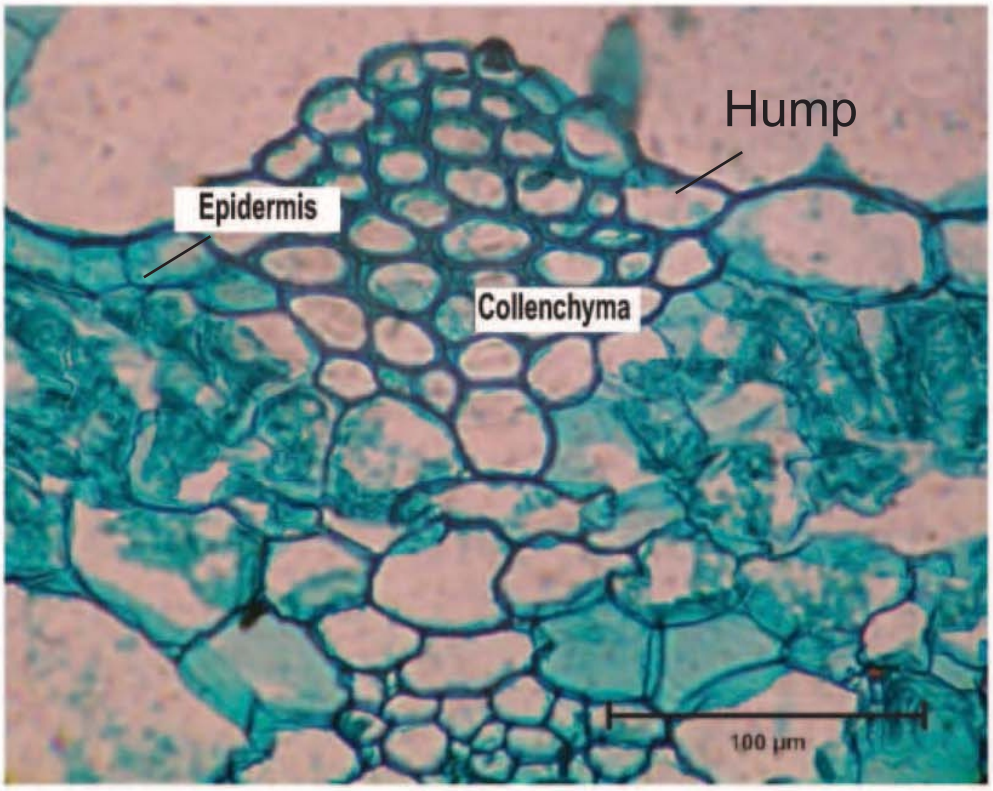


Plate - 9
Lower Epidermis (Showing Stomata)

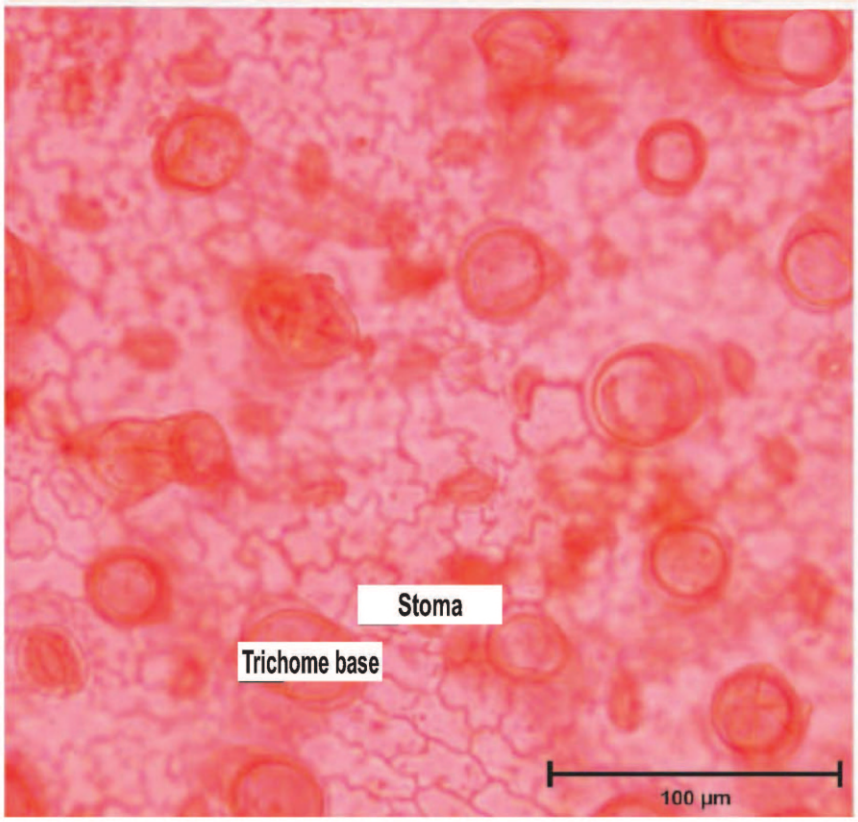


Plate - 9 A
Lower Epidermis- A portion Enlarged

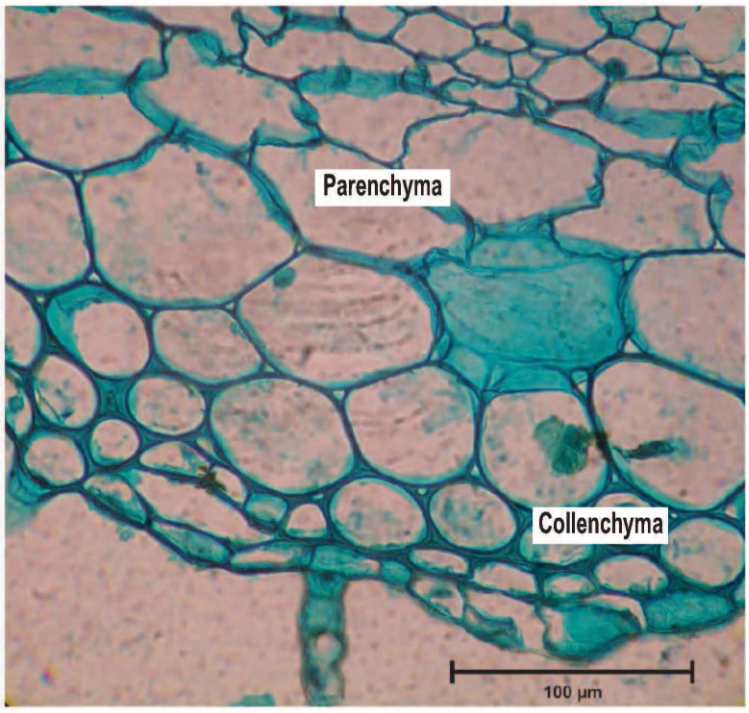
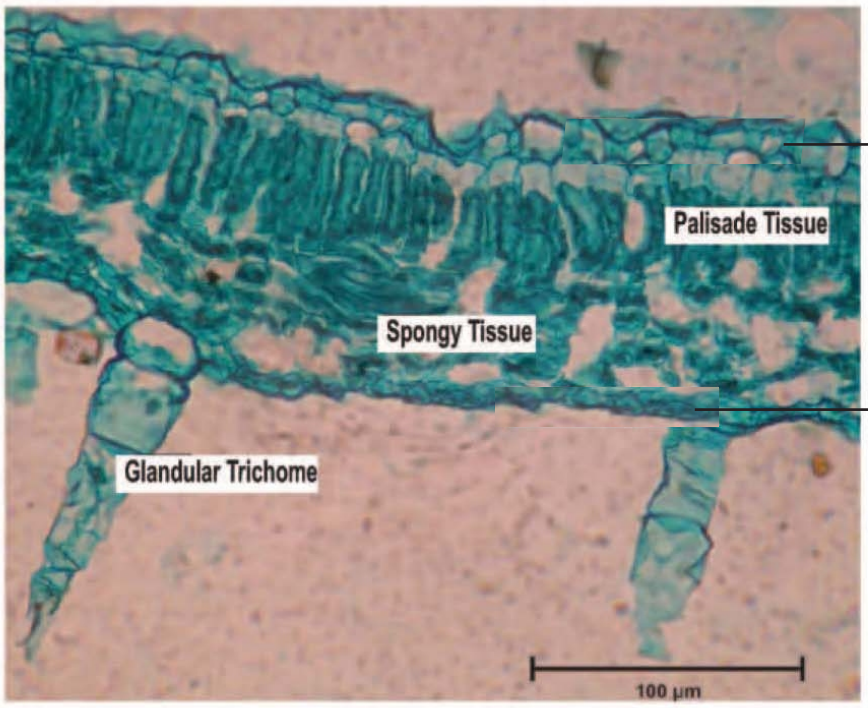


Plate - 10
T.S of Lamina



Lamina: (Plate 10)

The palisade tissue is composed of compact single layer of closely arranged Parenchyma cells and does not extend to the midrib region. The spongy tissue is made Up of round or oval 2-3 layers of parenchyma cells.

Trichomes: (Plate 10)

Numerous on both surfaces. Glandular hairs are uniseriate, long stalks of variable Length with multicellular spherical or disc shaped head. Non glandular, simple, Conical clothing hairs composed of 3-5 cells. Hair bases are seen as circular structure in both epidermis. Calcareous cystoliths occur at the base of the hairs or in adjacent epidermal cells. Vein terminals are rarly forked (Plate 11)

T.S of PETIOLE : (Plate 12,12A Fig-4)

Shape:

Oval wide deep adaxial depression in the centre with two humps on either Side and three ridges on the abaxial side.

Size:

2mm thick

Epidermis: (Plate 13,13A)

Single layered, rectangular cells covered with cuticle. Hypodermal regions contain 2 or 3 layers of collenchyma but below the humps and ridges they are 6-8 layers

Plate - 11
Venation Pattern

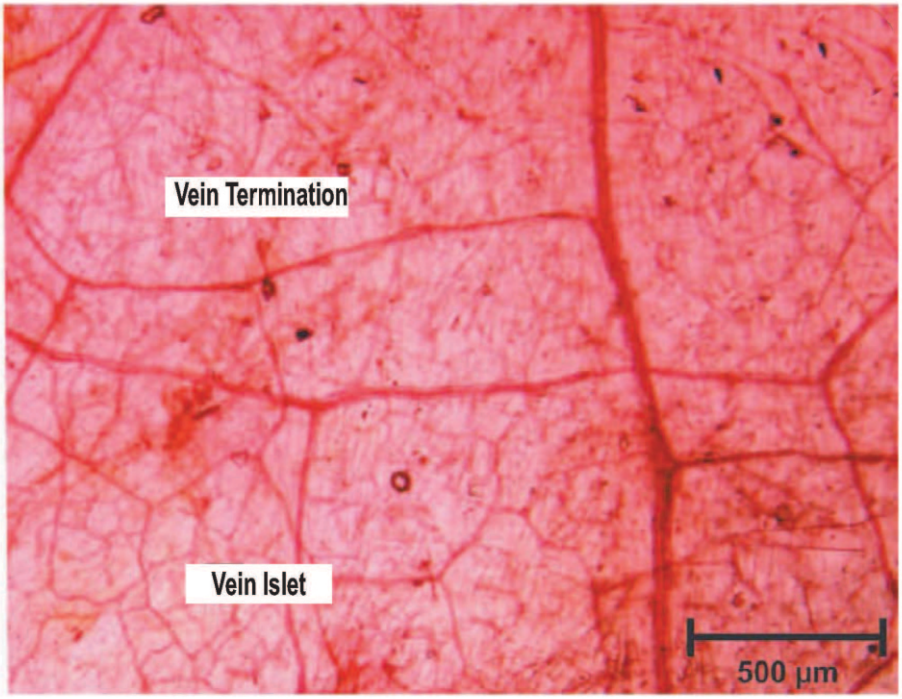


Plate - 12
T.S. of Petiole

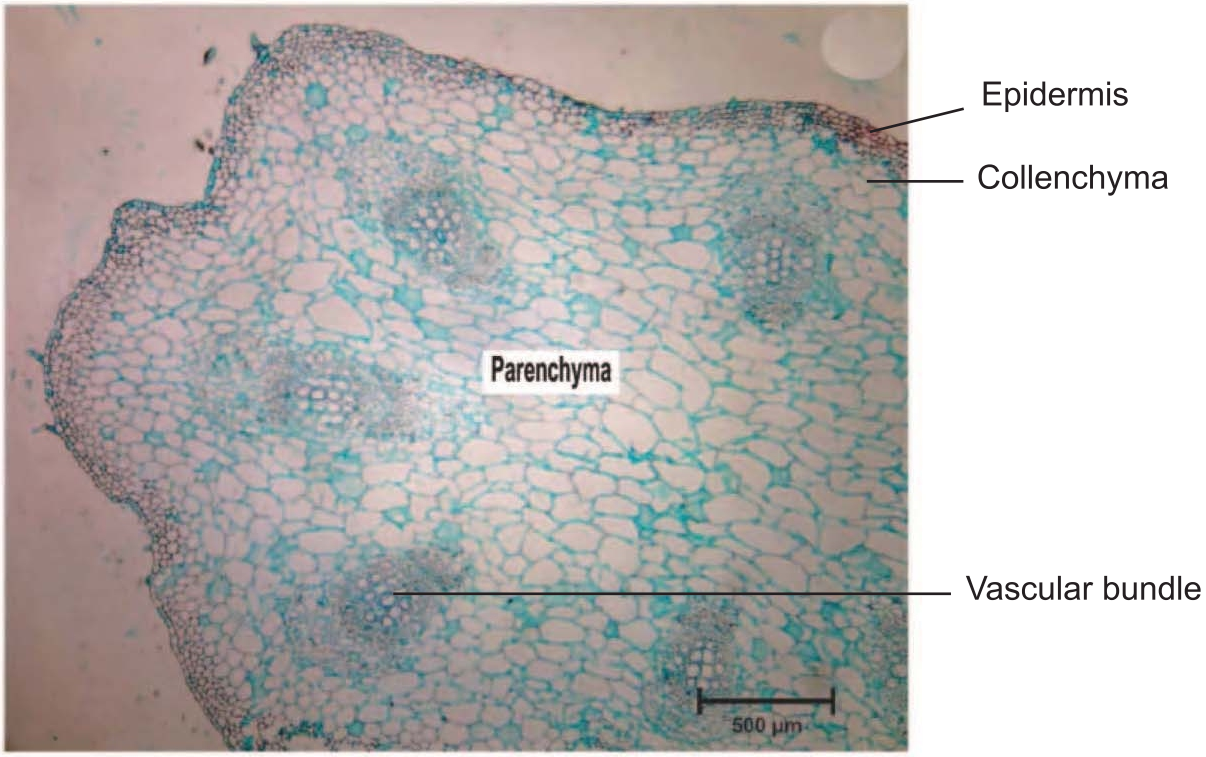


Plate - 12 A
T.S of Petiole- A Portion Enlarged

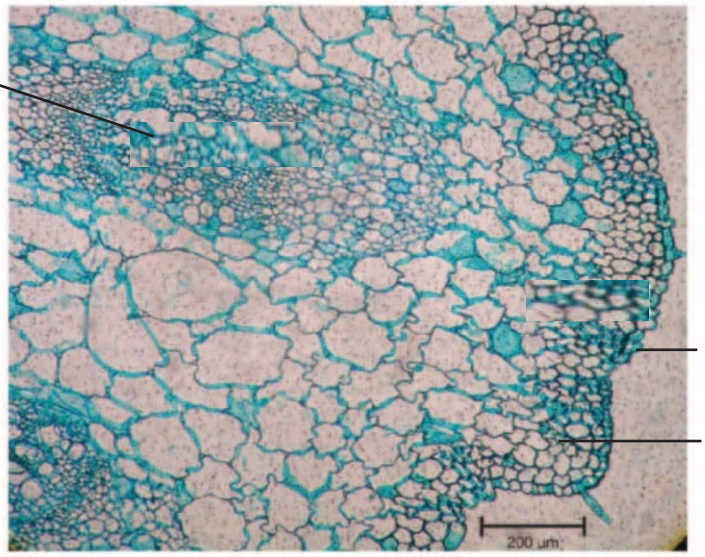


Plate - 13
T.S. of Petiole (showing humps)

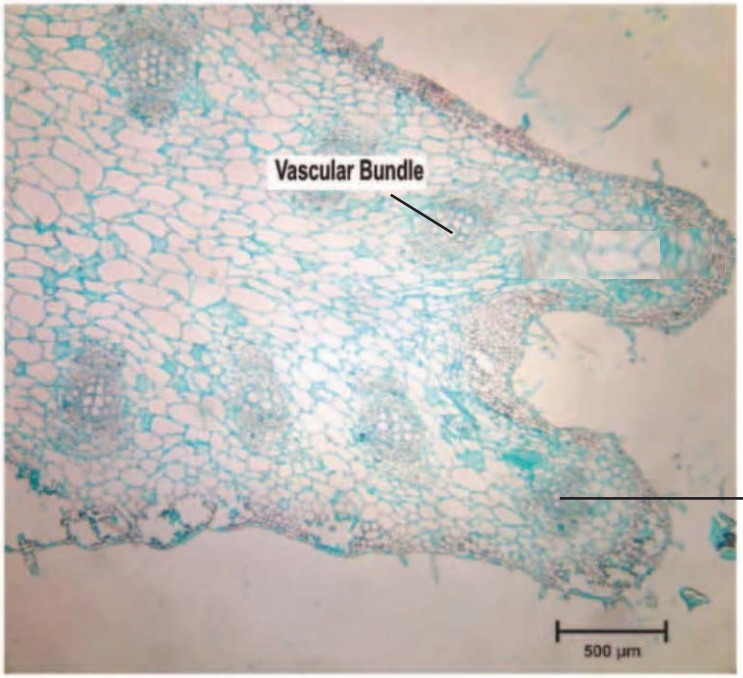


Plate - 13A
T.S. of Petiole hump - A portion Enlarged

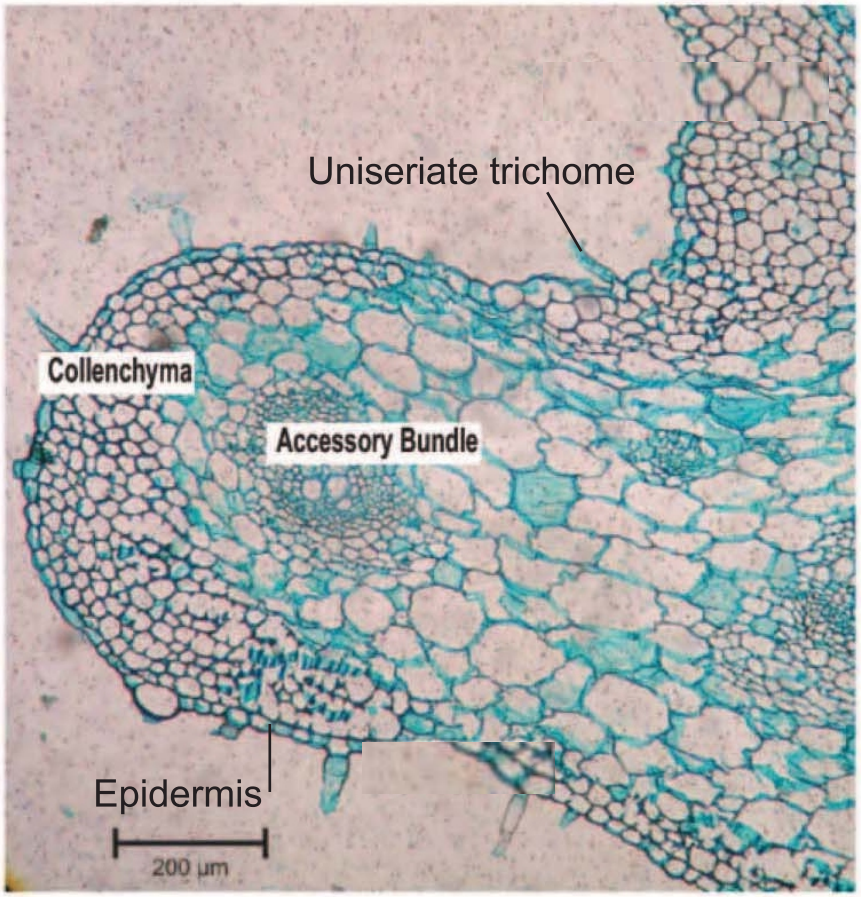
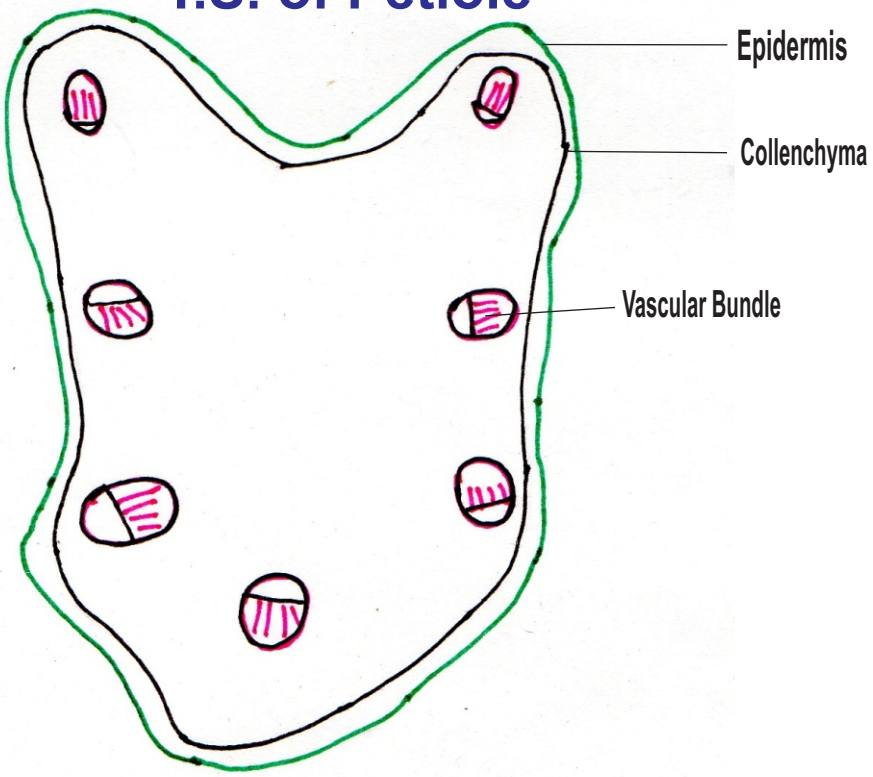
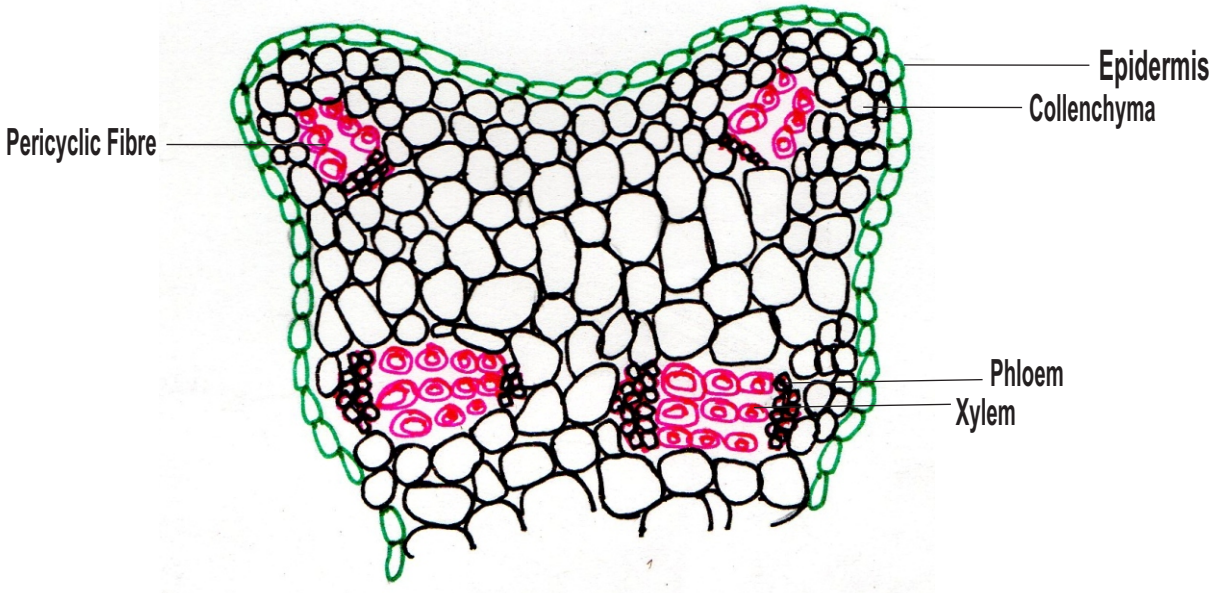


FIG - 4

T.S. of Petiole



T.S. of Petiole - A Portion Enlarged



Vascular bundles: (Plate 13-16)

Present in distal end as circle of collateral vascular bundles. They are separated from one another by broad strips of ground parenchyma. There are two smaller bundles one on each side of the adaxial hump.

5.1.3 SEM study of leaf (Plate 15)

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of microcostituents not previously recognised and apparently simple structure which may be extremely complex was observed.

5.1.4 POWDER MICROSCOPY (Fig -2)

Organoleptic characters

1. Nature : Coarse
2. Colour : Green
3. Odour : no characterictic odour.
4. Taste : no characteristic taste
5. Shaken with water : Froathing occurs
6. Pressed in between two filter paper : No oil mark on the paper

We have observed the following microscopical cell structures,

- Wavy walled epidermis
- Spiral,annular xylem vessels
- Pericyclic fibres
- ranunculaceous stomata
- Phloem cells(Sieve tubes with companion cells)
- Glandular and non glandular trichomes
- Collenchyma cells

FIG - 2

T.cucumerina Leaf Powder Microscopy

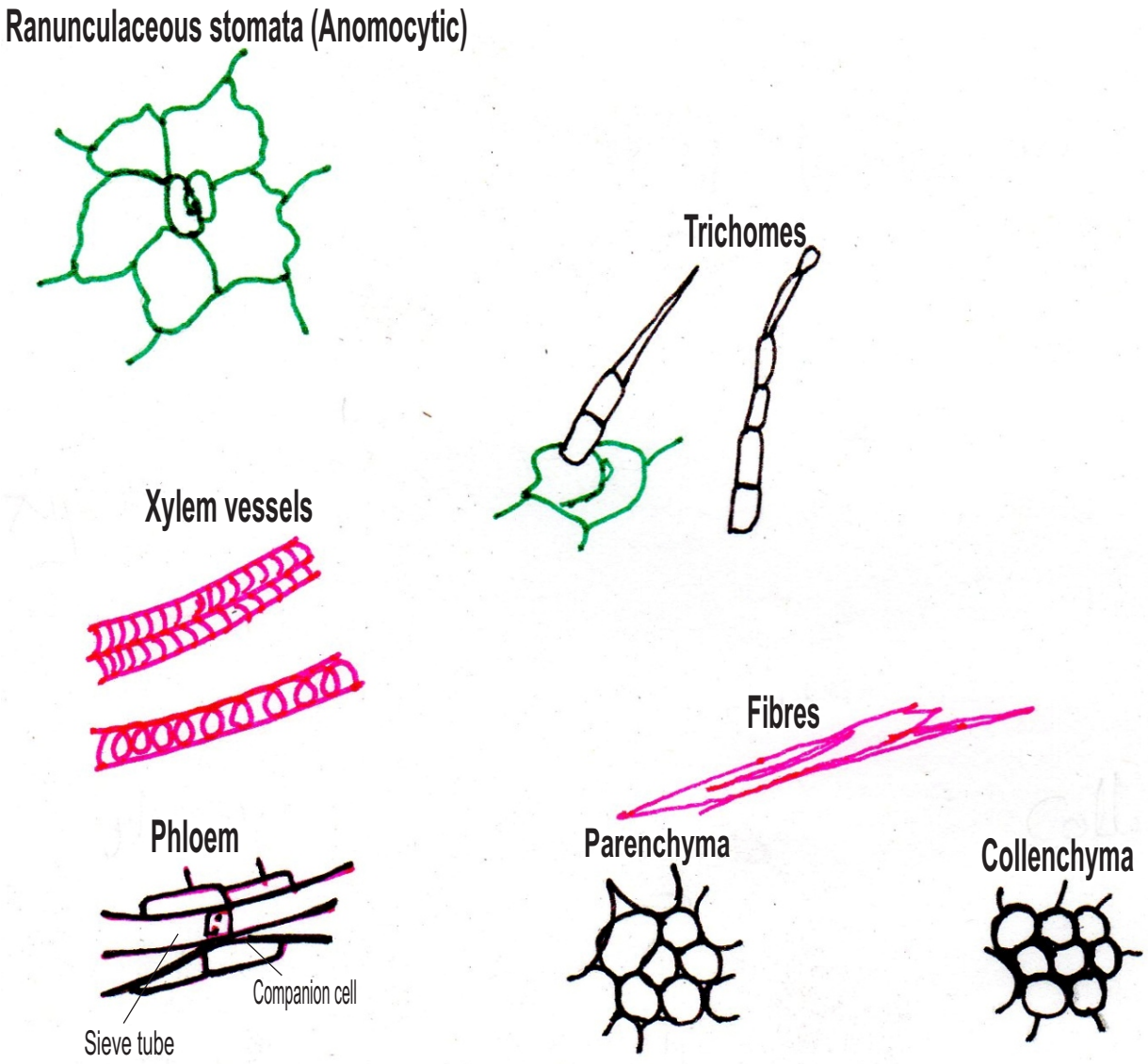
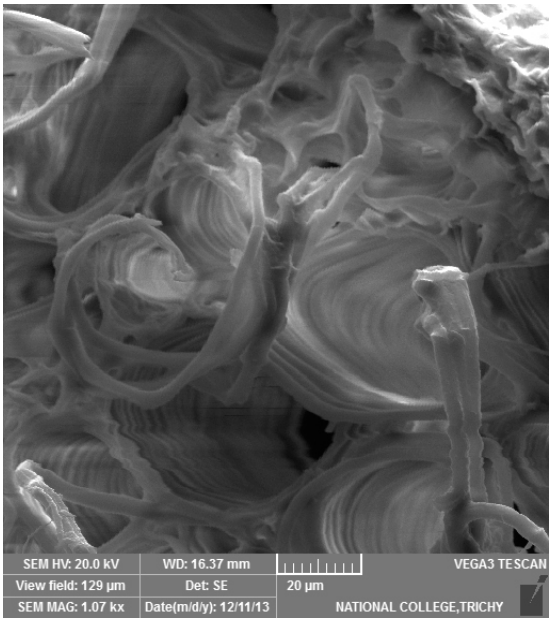
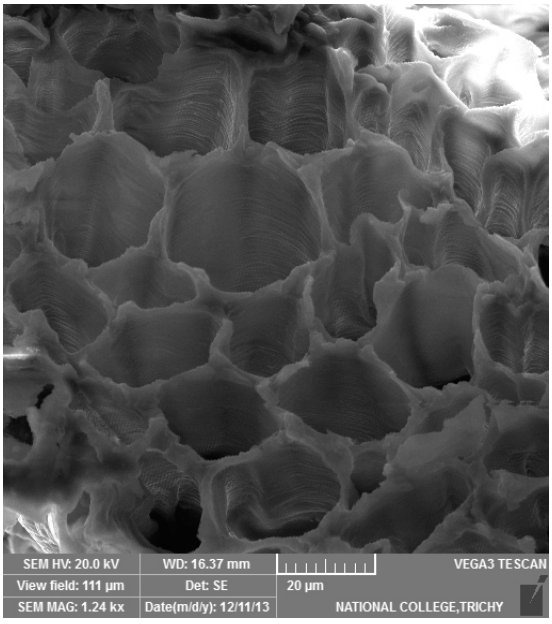


Plate 14

Vessels - Various Magnifications





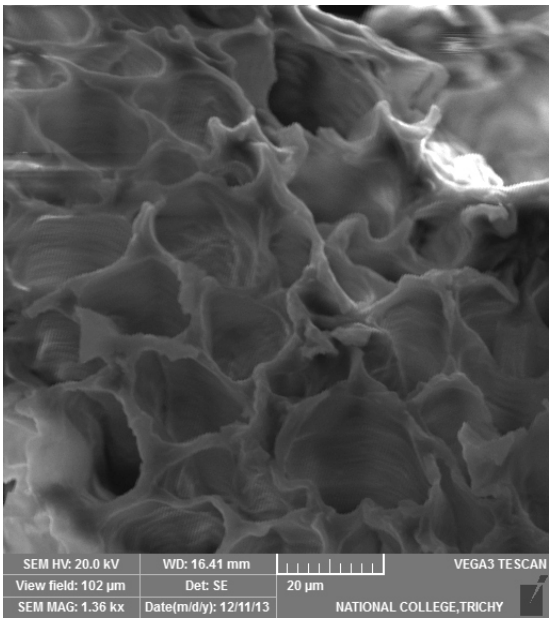
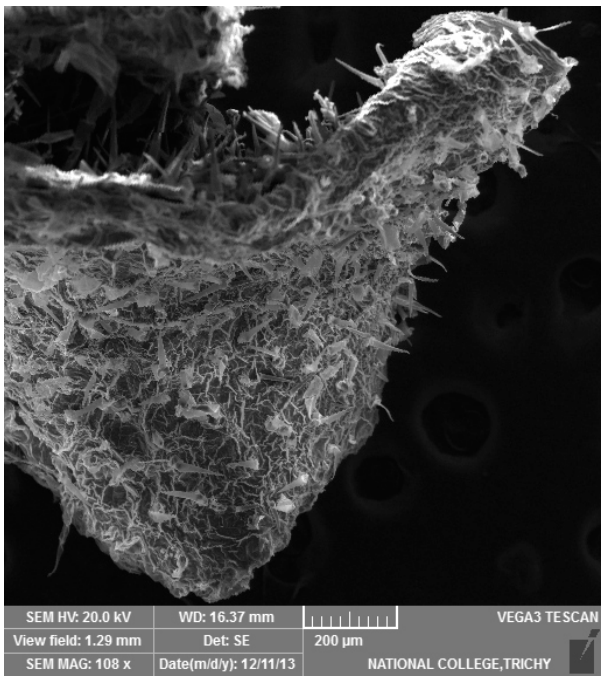
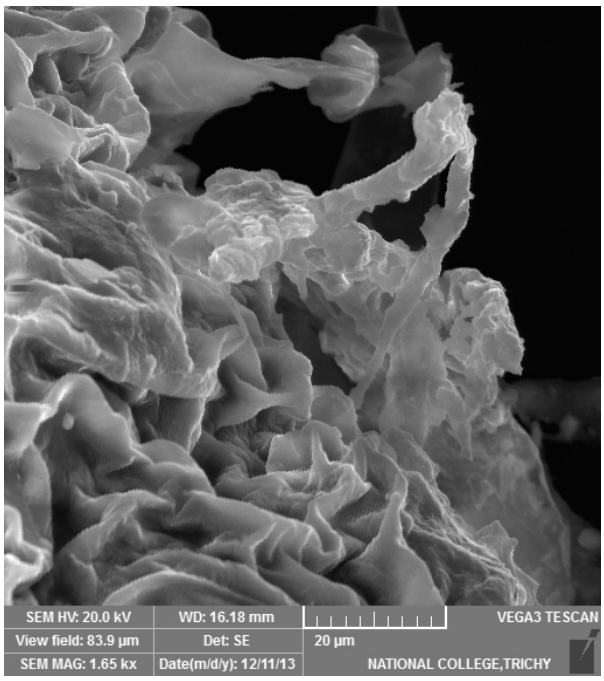
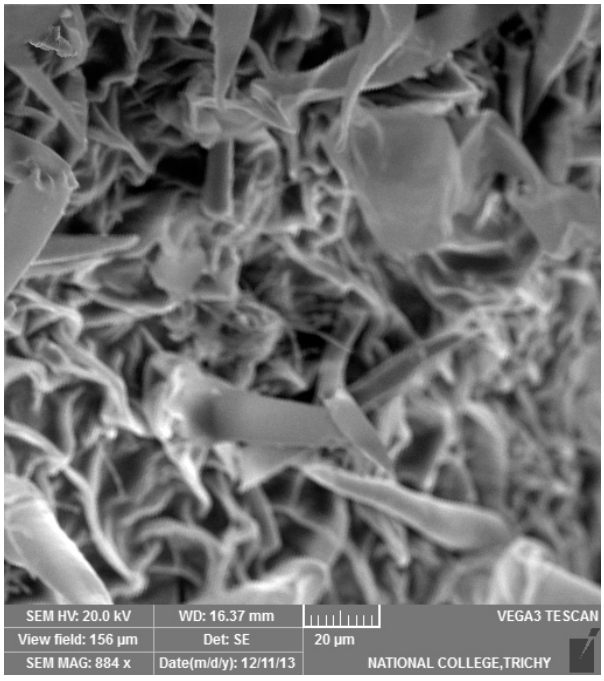


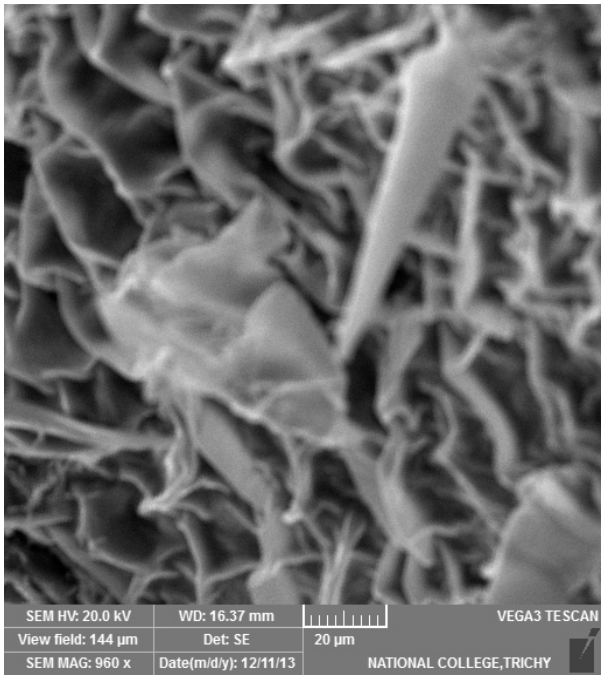
Plate 15

Epidermis, Trichomes - Under various magnifications









- Parenchyma cells

5.1.5 MICROSCOPIC SCHEDULE

As per the methods described in materials and methhods, microscopic schedules were carried out and the results tabulated from the Tables 1- 4.

Table – 1

VEIN ISLET AND VEIN TERMINATION NUMBER OF *T.cucumerina*

Observation number	Vein Islet number	Vein termination number
1	9	11
2	3	17
3	4	14
4	8	14
5	6	16
6	6	12
7	7	15
8	5	13
9	8	17
10	4	11

Range	Minimum	Average	Maximum
Vein islet number	3	6	9
Vein termination	11	14	17

Table – 2

STOMATAL NUMBER OF *T.cucumerina*(Lower Epidermis)

Observation number	Lower epidermis
1	27
2	26
3	19
4	28
5	20
6	21
7	24
8	29
9	27
10	25

Range	Minimum	Average	Maximum
Lower epidermis	19	24	29

Table – 3

STOMATAL INDEX OF *T.cucumerina*

Observation number	Lower epidermis
1	27
2	26
3	19
4	28
5	20
6	21
7	26
8	17
9	22
10	19

Range	Minimum	Average	Maximum
Lower epidermis	17	22.5	28

Table – 4

PALISADE RATIO OF *T.cucumerina*

Observation number	Palisade ratio
1	3
2	4
3	4
4	2
5	3
6	3
7	2
8	4
9	3
10	3

Minimum	Average	Maximum
2	3.125	4

5.1.6 PHYSICO CHEMICAL PARAMETERS

As per the methods described in materials and methods, physicochemical parameters were carried and the results were as follows

Table – 5

ASH VALUE OF THE LEAVES OF *T.cucumerina*

Observation Number	Total Ash (%)	Acid Insoluble Ash (%)	Water soluble Ash (%)
1	22.17	7.12	-
2	22.10	7.25	-
3	21.78	6.88	-
4	21.69	6.96	-
5	21.82	6.82	-
6	22.03	-	8.23
7	22.78	-	8.25
8	22.73	-	8.36
9	21.86	-	8.62
10	21.83	-	8.65
Minimum	21.78	6.82	8.65
Average	22.28	7.04	8.44
Maximum	22.78	7.25	8.23

Table – 6

LOSS ON DRYING (LOD) FOR THE LEAVES OF *T.cucumerina*

Observation number	LOD %w/w
1	7.39
2	7.04
3	7.56
4	7.12
5	7.15

Material	Minimum	Average	Maximum
Leaf powder	7.04	7.30	7.56

Table – 7

EXTRACTIVE VALUES (INDIVIDUAL SOLVENTS)

Solvents	Extractive value (%)
Petroleum ether	19.45
Ethanol	24.45
Water	4.54

Table – 8

EXTRACTIVE VALUES (SUCCESSIVE SOLVENTS)

Solvents	Extractive value (%)
Petroleum ether	19.45
Ethyl acetate	2.56
Ethanol	24.45
Water	4.54

5.2 PRELIMINARY PHYTOCHEMICAL SCREENING

5.2.1 Qualitative Phytochemical Test

Preliminary phytochemical screening of the powdered mature leaves were carried out and the results are as follows (Table 9)

TEST FOR ALKALOIDS

Mayer's test : No cream precipitate shows the **absence** of alkaloids

Dragendorff's test : No reddish brown precipitate shows the **absence** of alkaloids

Hager's test : No yellow precipitate shows the **absence** of alkaloids

Wagner's test : No reddish brown precipitate shows the **absence** of alkaloids

Muroxide test : No appearance of purple colour shows the **absence** of purine alkaloids

TEST FOR CARBOHYDRATES

Molish's test	:	Appearance of purple colour shows the **presence** of carbohydrates.
Fehling's test	:	Formation of reddish brown precipitate shows the **presence** of free reducing sugars.
Benedict's test	:	Formation of reddish brown precipitate shows the **presence** of free reducing sugars.

TEST FOR GLYCOSIDES

Keller killiani's test	:	No reddish brown colour ring at the junction shows the **absence** of cardiac glycosides.

TEST FOR PHYTO STEROLS

Salkowski's test	:	Appearance of red colour in lower layer shows the **Presence** of sterol
Liebermann – Burchard's test	:	Brown ring at the junction of two layers and green colour in the upper layer shows the **Presence** of sterols

TEST FOR SAPONINS

Frothing occurs indicates the **presence** of Saponins

TEST FOR TANNINS

Ferric chloride test	:	Appearance of bluish black colour shows the **presence** of tannins
Gold beater's skin	:	Appearance of brown colour shows the **presence** of tannins

TEST FOR PROTEINS AND FREE AMINOACIDS

Millon's test	:	Appearance of red colour on heating shows the **presence** of proteins

Biuret test : Appearance of violet colour shows the **presence** of proteins

Ninhydrin test : Formation of violet colour shows the **presence** of amino acids

TEST FOR MUCILAGE

Test : No appearance of reddish pink colour shows the **absence** of mucilage

TEST FOR TERPENOIDS

Test : Appearance of pink colour shows the **presence** of terpenoids

TEST FOR FLAVONOIDS

Shinoda test : Appearance of purple colour shows the **presence** of flavonoids

Alkaline reagent test : Appearance of yellow - orange colour shows the **presence** of flavonoids

Acid test : Appearance of yellow – orange colour shows the **presence** of flavonoids

Zinc hydrochloride test : Appearance of red colour shows the **presence** of flavonoids

TEST FOR VOLATILE OIL

Test : Volatile oil not obtained shows the **absence** of volatile oil

TEST FOR FIXED OIL

Test : No translucent greasy spot shows the **absence** of fixed oil

Table – 9

PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF *T.cucumerina*

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	-
	Dragondroff's reagent	-
	Hager's reagent	-
	Wagner's reagent	-
	Test for purine Group(Muroxide test)	-
II.	**CARBOHYDRATES**	
	Molisch's test	-
	Fehling's test	-
	Benedict's test	
III.	**GLYCOSIDES**	
	Anthroquinone glycosides	-
	Borntrager's test	-
	Modified Borndrager's test	-
	Cardiac glycosides	
	Keller Killiani test	-
	Raymond test	-
	Legal test	-
	Cyanogenetic glycosides	-
	Coumarin glycosides	-
IV.	**STEROLS**	
	Salkowski test	+
	Liberman Burchard's test	+
V.	**SAPONINS**	+
VI.	**TANNINS**	
	Ferric chloride	+
	Gold Beater's skin test	+
VII.	**PROTEINS AND FREE AMINO ACIDS**	
	Millon's test	+
	Biuret test	+
	Ninhydrin test	+
VIII.	**MUCILAGE**	-
IX.	**TERPENOIDS**	+
X.	**FLAVONOIDS**	
	Shinoda test	+
	Alkali test	+
	Acid test	+
	Zn/Hcl test	+
XI.	**VOLATILE OIL**	-
XII.	**FIXED OIL**	-

5.2.2 FLUORESCENCE ANALYSIS OF POWDERED LEAF

The fluorescence analysis of the leaf powder of *T.cucumerina* was studied. The results were as follows (Table -10)

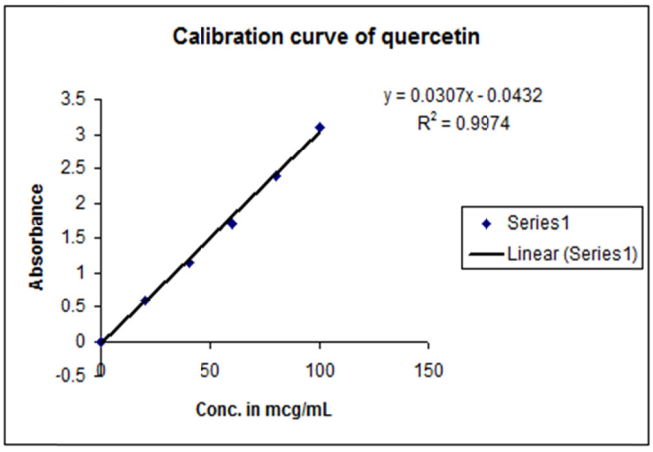
Table -10
FLUORESCENCE ANALYSIS

Reagent	Observation
Powder as such	Slight green
Powder + Conc Hydrochloric acid	Yellow
Powder + Conc Nitric acid	Reddish brown
Powder + Petroleum ether	Green
Powder + Conc Sulphuric acid	Brown
Powder + 5% NaOH	Yellow
Powder + 1N NaOH in methanol	Green
Powder +5% Ferric chloride solution	Brown
Powder +50% Picric acid	Green
Powder + Chloroform	Light Green
Powder + 5% Iodine solution	Dark Green
Powder + (HNO3 + NH3)	Green

5.2.3 Estimation of flavonoid content of TCEAE

Figure - 5

Calibration curve of quercetin for estimation of flavonoid content



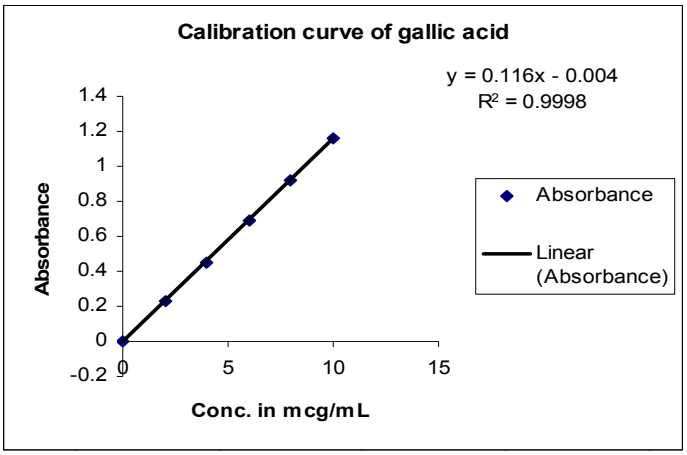
Report:

Flavonoid content of TCEAE in terms of quercetin by aluminium chloride was found to be 219 mg/g.

5.2.4 Estimation of Total phenolic content of TCEAE

Figure - 6

Calibration curve of gallic acid for estimation of total phenolic content

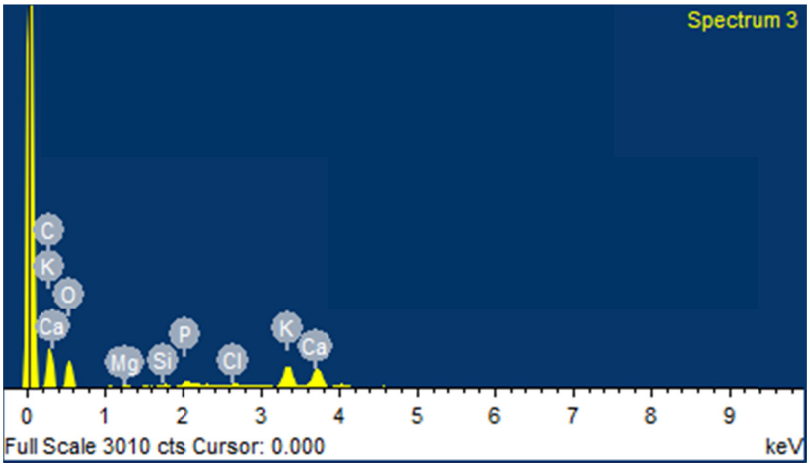


Report:

Total phenolic content of TCEAE in terms of gallic acid was found to be 376.5 mg/g.

5.2.5 IDENTIFICATION OF INORGANIC MINERALS OF THE LEAVES OF *T.cucumerina* by Energy Dispersive X-ray Spectrometer (EDS) (Plate)

Figure-7



Estimation of the elements like C, O, Mg, Si, P, Cl, K, Ca showed the following mg weight percentage and atomic percentage.

Table-13

SI.NO	ELEMENTS	WEIGHT(%)	ATOMIC(%)
1.	CK	43.59	54.68
2.	OK	41.95	39.50
3.	MgK	0.51	0.32
4.	SiK	0.35	0.19
5.	PK	1.04	0.51
6.	ClK	0.80	0.34
7.	KK	5.91	2.28
8.	CaK	5.84	2.20

5.2.6. HPTLC PROFILE OF THE TCEAE &TCEE OF THE LEAVES

Development of HPTLC fingerprint

About 2,6,8,10 µl of standard Apigenin ,4,8 µl of TCEAE,3 µl Quercetin,6µl of TCEE was applied as a band using CAMAG Linomat sample applicator on aluminium sheets pre-coated with silica gel 60 GF 254 HPTLC plates used as a stationary phase. The plates were developed in the mobile phase Toluene : Ethyl acetate : Formic acid : Methanol (3:6:1.6:0.4) to a distance of 8cm in CAMAG-twin trough glass chamber. The tracks were scanned using WIN CATS 1.43 software at 254nm. The fingerprint profiles were recorded and presented in **Table-14**

Plate size : HPTLC plates silica gel 60 F 254

(E.MERCK KGa A)

Sample solvent type : Methanol

Dosage speed : 150 nl/s

Number of tracks : 8

Table-14

NO	Appl.position	Appl.volume	Sample ID	Active
>1	10.0 mm	2.0 µl	API	Yes
>2	21.4 mm	6.0 µl	API	Yes
>3	32.8 mm	8.0 µl	API	Yes
>4	44.2 mm	10.0 µl	API	Yes
>5	55.6 mm	4.0 µl	TCEAE	Yes
>6	67.0 mm	8.0 µl	TCEAE	Yes
>7	78.4 mm	3.0 µl	QUERCETIN	Yes
>8	89.8 mm	6.0 µl	TCEE	Yes

Chamber type : Twin Trough Chamber 10×10cm

Mobile phase : TOLUENE: ETHYL ACETATE:FORMIC ACID:METHANOL (3:6:1.6:0.4)

Drying device : Oven

Temperature : 60□ C

Time : 5 Minutes

Detection : CAMAG TLC Scanner 3

Scanning speed : 20 mm/s

Wavelength : 254

Lamp : D2 &W

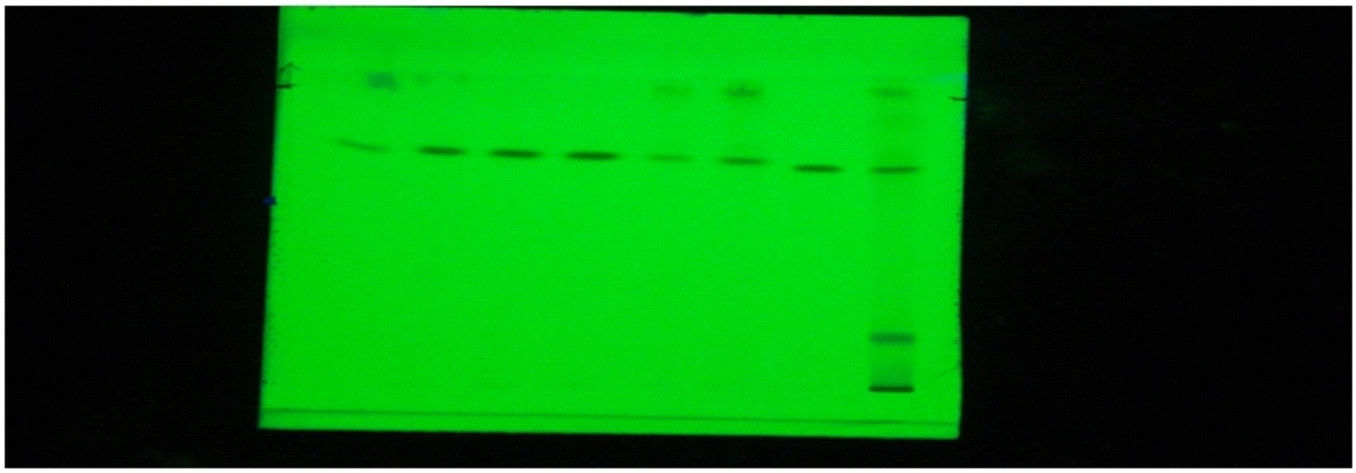
Measurement Type : Remission

Measurement Mode : Absorption

Detector mode : Automatic

Plate 16

HPTLC PLATES OF TCEAE AND TCEE OF THE LEAVES UNDER UV 254nm



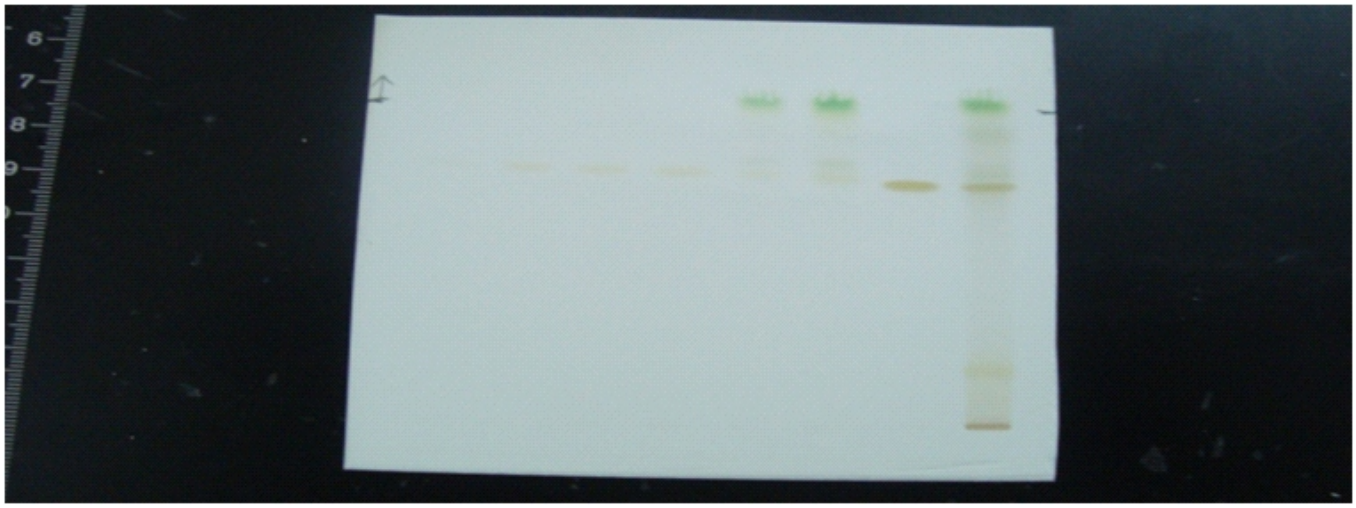
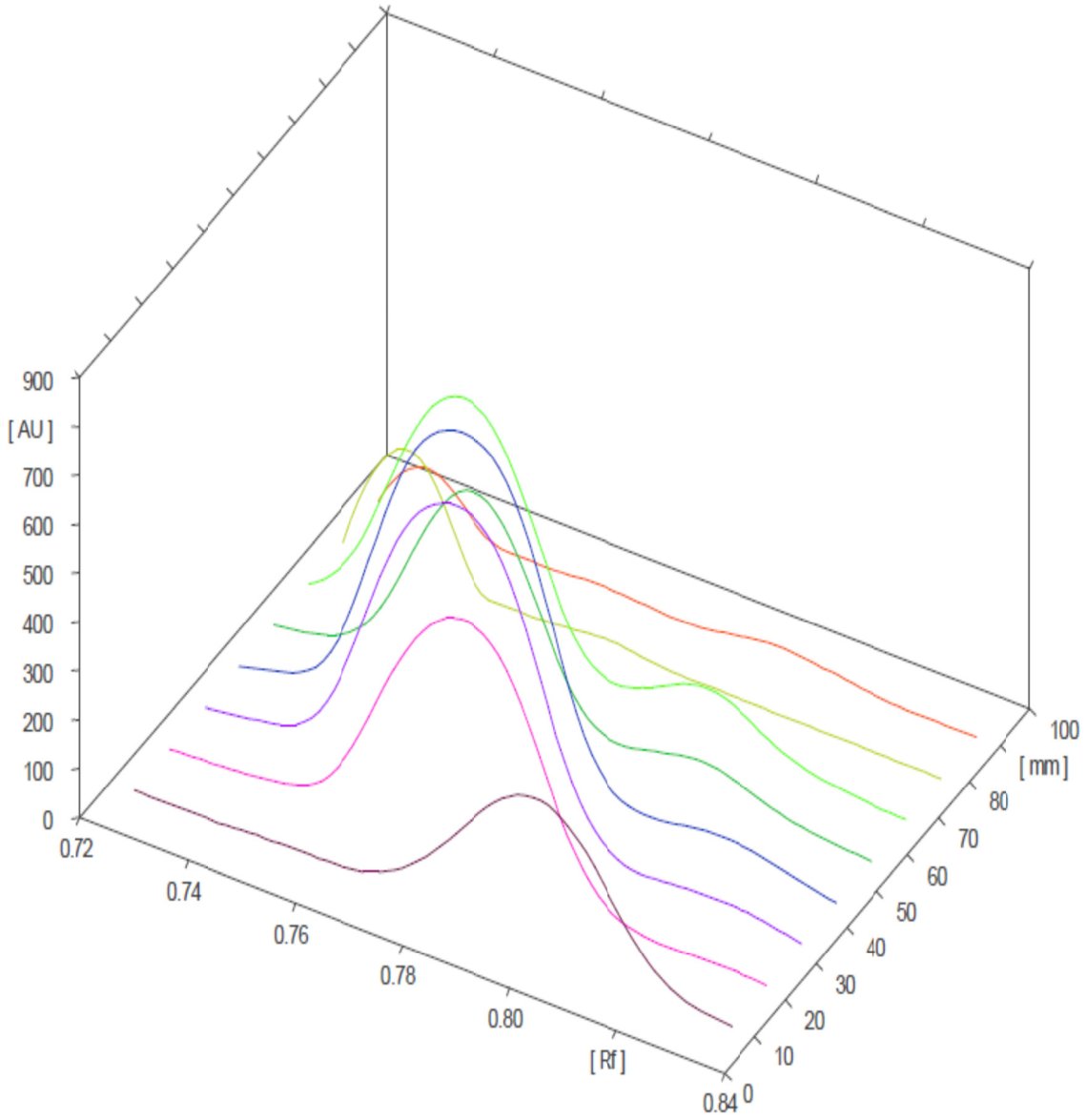
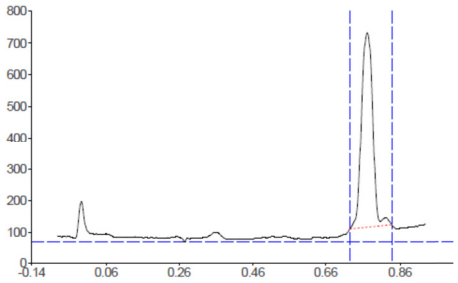


Figure-8

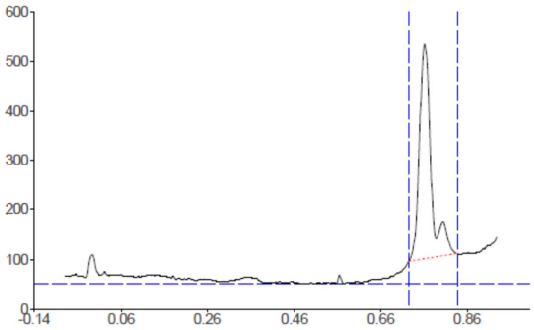
HPTLC PROFILE OF THE TCEAE AND TCEE OF THE LEAVES



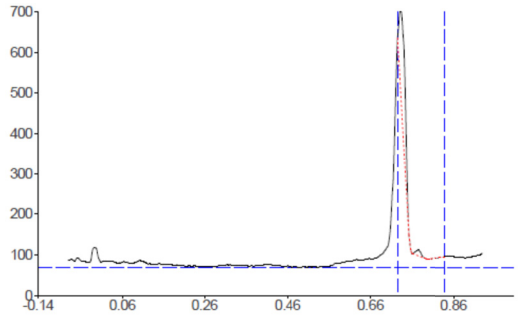
STANDARD : APIGENIN



STANDARD : QUERCETIN



SAMPLE : TCEAE



SAMPLE : TCEE

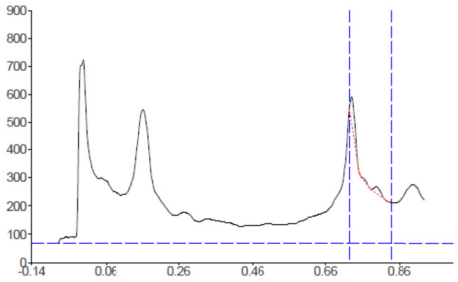


Table - 15

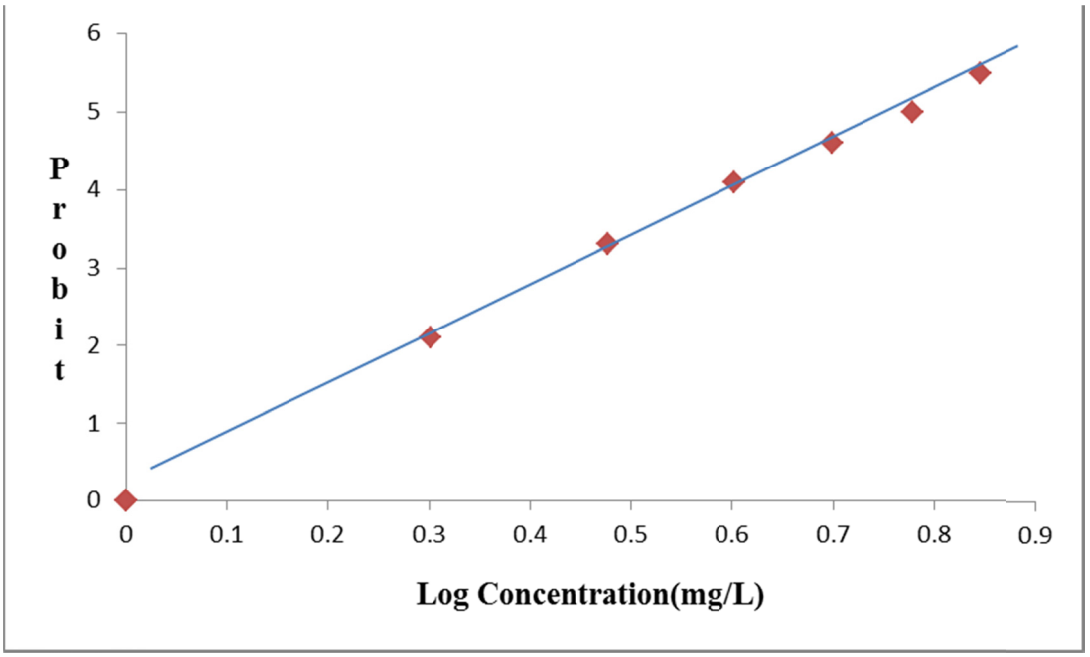
SI NO	SAMPLES	Start Rf	Max Rf	Max %	End Rf	Area	Area %
1	Standard Apigenin	0.73	0.77	96.34	0.81	14882.2	97.62
2	Sample TCEAE	0.73	0.76	86.48	0.79	8461.9	88.02
3	Standard Quercetin	0.73	0.74	93.99	0.75	2385.0	94.36
4	Sample TCEE	0.73	0.73	79.76	0.75	723.2	66.35

5.3. PHARMACOLOGICAL STUDIES

5.3.1. Assessment of acute toxicity of TCEAE leaves on *D.magna*

We assessed the acute toxicity of TCEAE leaves using daphnids. Percentage mortality or immobility of control (spring water), test drug TCEAE (1, 2, 3, 4, 5, 6mg/L) were observed. No mortality was observed in control. Gradual increased rate of mortality was observed with increasing concentration of TCEAE. The readings were plotted log concentration in X-axis against probit in Y-axis (Figure-8). From the graph LC_{50} value was calculated and found to be **5.88mg/L**.

LC_{50} of TCEAE



5.3.2. EVALUATION OF EFFECT OF TCEAE ON LACTOSE INDUCED ARRHYTHMIC HEART OF *Daphnia magna*

Cardiac arrhythmia induced by lactose (200Mm). After 30 minutes the heart beat of various concentration of test drug TCEAE (20, 40, 60, 80 µg/ml), standard drug metoprolol (20, 25 µg/ml) in triplicate on lactose induced arrhythmic heart of *D.magna* were observed under the microscope. Readings were plotted concentration

in X-axis vs heart beat (bpm) in Y-axis (Figure-9). The result was statistically significant ($p<0.05$)

5.3.3.EFFECT OF TCEAE LEAVES ON *ex-vivo* PORCINE SKIN WOUND HEALING MODEL:

Wound healing evaluated by *ex-vivo* porcine skin wound healing model (PSWHM). Architecture of pig skin is closer to human (Bollen Peter JA *et al*, 1999, Laber *et al*, 2002). Porcine model is an excellent tool for the evaluation of therapeutic agent meant for wound healing (Sullivan TP *et al*, 2001).

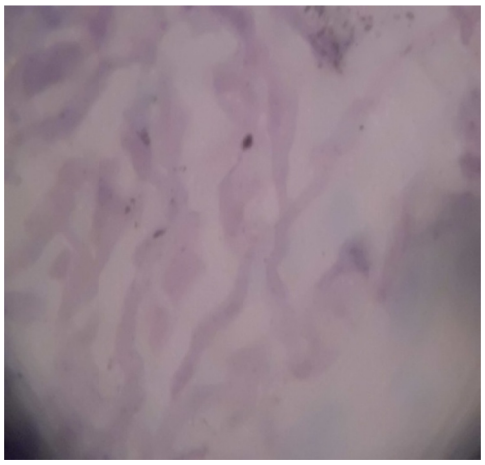
Histopathological evaluation showed all treated wounds were sound with no signs of apoptosis, necrosis or bacterial contamination and no toxicity of the tested concentrations of TCEAE of the leaves.(Plate 16). Morphology of the wound margins, epidermis and dermis layer were found to be normal. Epidermal cell migration in each side of the wound was measured from the edge of the wound to the migration tip.

Measurement of epidermal migration distances from the wound showed statistically significant dose dependent wound healing effect (Anova $p<0.05$).The efficacy of wound healing promoting effect was expressed as normalised epidermal migration % mean SE against PBS control groups.(Fig 10)

PLATE-17

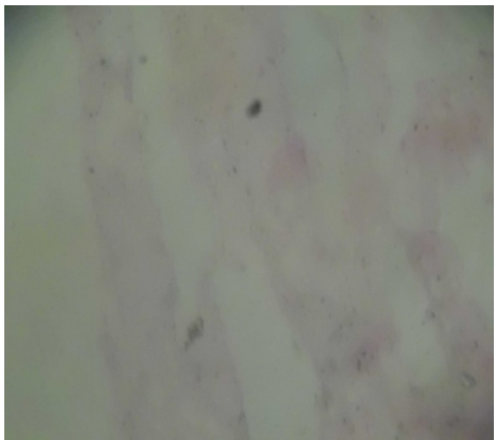
HISTOLOGY SHOWING EFFECT OF TCEAE ON *ex-vivo* PORCINE SKIN WOUND HEALING MODEL(PSWHM)

CONTROL



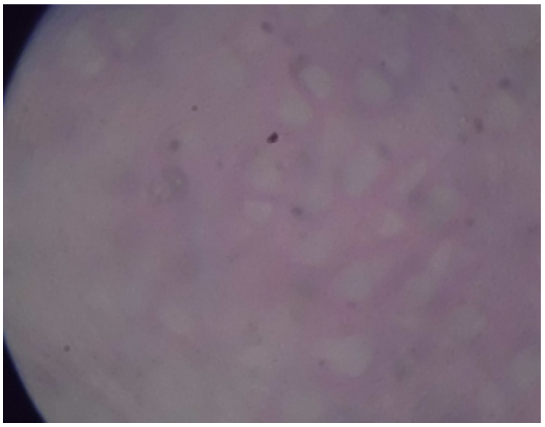
1% OF TCEAE

2% OF TCEAE



3%TCEAE

STANDARD(MUPIROCIN)



CHAPTER-6

DISCUSSION

CHAPTER - 6

DISCUSSION

The dissertation covers a study on the widely available a member of the family Cucurbitaceae is known botanically as *Trichosanthes cucumerina*,Linn. commonly called as snakegourd. The leaves of *Trichosanthes cucumerina* really do not have any match as a cheap natural and easily available plant. It is traditionally known to be useful for the treatment of wide panel of diseases like diabetes,wounds,skin diseaeses, alopecia, diarrhoea, haematuria, malaria, bronchitis, refrigerant, liver diseases etc. Antihepatotoxic, antifertility, hepatoprotective activity, anti-inflammatory, antidiabetic, antiulcer and antibacterial, diuretic, antioxidant, antihistamine, gastroprotective activities have been studied and found effective. Leaf is used as cardiotonic, anti pyretic, antiperiodic, emetic, antihelmintic, and externally applied over bald patches of alopecia. Juice is rubbed over the liver in liver congestion and all over the body in remittent fever. Hair growth promoting activity, anti bacterial activity, larvicidal activity and toxicity studies have been performed and found effective and safe. Fruit used as an antioxidant, anti-inflammatory, antibacterial, anticariogenic, antifungal, hypoglycemic, anti diabetic and excellent source of fibres, vitamins, minerals and proteins. Anticancer activity, cardioprotective activity, antioxidant activities and toxicity studies have been investigated and found effective and safe. Seed is used as cooling, as anthelmintic, antidiarrhoeal, abortifacient, aphrodisiac, astringent, febrifuge. Cardioprotective, antioxidant, antibacterial, antispasmodic, insecticidal, antidiabetic, anti febrile, actions have been studied. Roots useful in treating diabetes, skin swelling,convulsion. Anticancer activity was carried out and found effective. This plant is much more popular in India and widely

cultivated. In India fruits used as food and is rich source of nutrition constituted with proteins, fat, fibre, carbohydrates, vitamin A & E, ascorbic acid, carotenoids, lycopene,flavonoids, carotenoids, phenolic acids and minerals like potassium, phosphorous, magnesium, zinc etc. The economic aspect of this crop evidently proved that as commercial crop. In fact the revenue generated by this crop can be further magnified by many folds, if its medicinal applications are scientifically explored well. By a well-coordinated effort, we can properly exploit this plant. Therefore research on development of herbal products from this plant is required to be initiated immediately for exploring the unique potential of this crop which would also minimize the menacing wastage especially the leaves. It may be further envisaged that the revenue generated by this plant would easily exceed that generated by any major crop of the country even with a present level of traditional agro economic practices. Therefore a well-coordinated effort by the farmers, traders, scientist, technologists, extension workers, physician, administrators, and policy makers is required to be initiated to boost up the national economy as well as the proper exploitation of this for proper therapeutic purpose. The review of literature showed some lacunae exists in the pharmacological, phytochemical, and pharmacological studies in the leaves of *T.cucumerina*.

PHARMACOGNOSTICAL STUDIES:

Morphological and micromorphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacological studies. There was no detailed pharmacognostical work has been carried out including botanical identity based on micro morphology in this leaves of this plant.

The application of morphological studies in drug analysis is pertinent in the field of crude drug authentication. It was studied for the leaf. Interpretation of the morphological characteristics based on different parameters, for the plant organs give a guideline for the diagnosis of the original plant and its adulterants.

Colour, size, shape, margin, texture, arrangement were observed and compared with previous data.

Microscopic techniques help to magnify the fine structure of minute objects and there by confirm the structural details of the plant drug. Though the microscopical evaluation cannot provide complete profile, still it can offer supporting evidences which when combined with other analytical parameters can be used to obtain full evidence for standardization and evaluation of herbal drugs. Consideration must therefore be given to the types of cells and cell inclusions and the manner in which they are distributed in different organ of the plants. Leaves are dorsiventral with prominent midrib with various shapes lobed reniform. In transactional view it is planoconvex shape with slightly elevated in the centre of adaxial side, convex, broad abaxial side, Vascular bundle. The bundles contain sheath of phloem in the outer part. Numerous uniseriate multicellular glandular and nonglandular covering trichomes are present on both sides.. The upper epidermis is apostomatic and the lower epidermis contains ranunculaceous stomata, broadly elliptical with long, narrow aperture. Collenchyma is present beneath upper and lowerepidermis.The petiole oval, wide deep adaxial depression in the centre with two humps on either Side and three ridges on the abaxial side. Epidermis Single layered, rectangular cells covered with cuticle. Hypodermal regions contain 2 or 3 layers of collenchyma but below the humps and ridges they are 6-8 layers. Vascular bundles: present in distal end as circle

of collateral vascular bundles. They are separated from one another by broad strips of ground parenchyma. There are two smaller bundles one on each side of the adaxial hump.

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of microcostituents not previously recognised and apparently simple structure which may be extremly complex was observed (Plate-7-15).

The plant drugs are generally used in the powdered form where the macro morphology is generally destroyed, so the diagnosis of the plant through the microscopical character is essential. The powdered crude drugs can be identified based on the presence or absence of different cell types. In powdered microscopy, we have observed wavy walled epidermal cells, spiral xylem vessels, ranunculaceous stomata, glandular and non glandular covering trichomes, phloem cells with sieve tubes and companion cells, pericyclic fibes,

Quantitative microscopy includes certain measurements to distinguish some closely related species which are not easily differentiated by general microscopy. The **stomatal number** is the oldest technique but a simple method of diagnosis of fragmentary leaf parts. The stomatal index is the percentage of stomata in relation to the epidermal cells. Both are very specific criteria for the identification and characterization of leafy drugs. **Vein islet and vein termination number** are another simple technique for distinguishing fragmentary specimens at specific levels. It is used as the distinguishing character for the leaf of the same species or different one.

Palisade Ratio is another criterion for identification and evaluation of herbal drugs. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value does not alter based on geographical variation and differs from species to species and that is why it is a very useful diagnostic feature for characterization and identification of different plant species. (Table 1-4)

The ash content of the crude drug is generally taken to be the residue remaining after incineration. If usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also involve the inorganic matter added for the purpose of adulteration. There is a considerable difference within narrow limits in the case of individual drug. Hence ash determination furnishes a basis for judging the identify and cleanliness of a drug and gives information related to its adulteration with inorganic matter. The ash or residue yielded by an organic chemical compound is a rule to measure the amount of inorganic matter, which is present as impurity. In most cases the inorganic matter is present in small amounts which are difficult to remove in the purification process and which are not objectionable if only traces are present. Ash values are helpful in determining the quality and purity of the crude drug in especially in powdered from. The **acid insoluble ash** is of more value to detect the earthy matter adhering to the drug. In this way one can obtain evidence of the presence of foreign matter, which likely to occur with root, rhizomes and also in pubescent leaves. **The water soluble ash** is used to detect the presence of matter exhausted by water. Insufficient drying favours spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles (Table -5).

Extractive values of crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books (Anonymous 1996, BP 1980, BHP 1990 etc.), the determination of water-soluble and alcohol soluble extractive, is used as means of evaluating crude drugs which are not readily estimated by other means. The extraction of any crude with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The **water soluble extractive** values play an important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. The **alcohol soluble extractive** is also indicative for the same purpose as water soluble extractive values (Table -7, 8).

Loss on drying is determined as the presence of excess moisture is conductive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug (Table -6)

THE PRELIMINARY PHYTOCHEMICAL STUDIES:

The preliminary phytochemical screening reveals the presence of carbohydrates, proteins and amino acids, flavonoids, terpenoids, tannins, saponin, and phytosterols. Alkaloids, volatile oil, fixed oil were found to be absent. (Table 9)

The reaction of drugs in powdered form in ordinary light and with filtered UV light is of importance in several cases by the luminosity in UV light by **fluorescent analysis**. Many flavonoids showed distinctive colours under UV light: Bright yellow (6-hydroxy flavanoids and flavones and some chalcones), dark brown (most flavanol glycosides, dark may be (isoflavones and flavonols). Hence this parameter can also be used as a diagnostic tool for the standardization of herbal drugs for the detection of adulterants in crude drugs (Table – 10) (Harborne JB 1973).

Total flavonoid content of TCEAE was determined as quercetin equivalent and found to be 29mg/g and Total phenolic content of TCEAE in terms of gallic acid was found to be 76.5 mg/g.

Identification of inorganic minerals of the leaves of T.Cucumerina by Electron dispersive x-ray Spector photo meter(EDS) showed the presence of minerals Calcium (5.84%), Potassium (5.91%), Magnesium (0.5%), chlorine (0.8%),(Table-13).

HPTLC profile of TCEAE was performed and the presence of apigenin and quercetin was identified and quantified. TCEAE contains apigenin and quercetin 0.77,20% respectively(Table 14)

PHARMACOLOGICAL STUDIES:

Plants have an almost limitless ability to produce aromatic substances, most of which are phenols or their oxygen substituted derivatives. In many case these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some like terpenoids give plants their odours other are responsible for plant pigments. The phenolics and its derivatives were reported to be effective against viruses, bacteria and fungi, and many diseases including wound

healing. The above points prompted us to investigate ethyl acetate extract from the leaves of *T.cucumerina* a widely available and menacingly wasted part of it. Its medicinal applications are still to be explored well. So we have initiated a research for exploring the unique potential of the leaves of TCEAE to minimize the menacing wastage and to maximize the revenue generated by this crop to boost up our national economy as well as the proper exploitation of this plant for therapeutic purpose. By investigating the bioactivity of TCEAE, we can meet the situation of unsettling facts of modern pharmaceutical industry which facing lately its pipeline of new drug discovery seems to be almost empty**.**

We planned to screen the cardio protective of TCEAE leaves against lactose induced arrhythmia using *Daphnia magna* as a model organism along with acute toxicity assessment.

Advantage of *D. magna* screening :(Navarro AV *et al*, 2003, Schleidt S *et al*, 2009, Kass B *et al*, 2009)

1. Genomic sequence of *D.magna* shares most with humans.
2. Its myogenic heart, while most arthropods hearts are neurogenic.
3. Transparent carapace allows easy observation of heart. So apply optical methods to visualize physiological function and to measure several different parameters simultaneously.
4. Non-invasive method.
5. Drugs are directly added to the water in which they swim.
6. In concern of animal welfare, no need of ethical clearance.
7. *D. magna* has similar ANS in compared to humans.
8. Economically feasible. Easy to culture in lab.

9. Less time consuming
10. More sensitive, accurate results.

The **3R's** ethical principle (**R**eduction, **R**efinement, and **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science. We planned to carry out both *in vivo* and *ex-vivo* study without using mammalian system in this drug discovery process and also to help cut cost and save time.

Various problems are inherent during the use of animal models such as high cost of housing animals and ethical issues which has become more serious in recent years. Law strictly regulates the use of mammals for the development of medicine.

As the small animal models are emerging, it is now possible to perform *in vivo* testing. So researchers has developed animal model system using both vertebrates like zebrafish and invertebrate models like fly*, Drosophilla melanogaster*, *D.magna*,etc for drug screening. These are very powerful tools for identifying host protein involved in immune system because they are genetically tractable and many mutant lines have been constructed. So we have preferred *D.magna* for studying cardio protective and the therapeutic effects. There are no ethical problems and biohazards associated in the use of a large number of *D.magna.*

Hence low cost, no ethical issues, its transparent myogenic heart, possible to count the heart beat and no biohazards were attracted us to undertake this study *in vivo* using *D.magna* to screen cardio protective studies.

It shares myogenic heart, common ANS property on heart with mammals when we review the previous reports. Therefore, the water flea model is potentially useful for the evaluation of toxicity of candidate compounds in therapeutic drug

screening for arrhythmia. Furthermore, *Daphnia* are large enough to count its heart beat (bpm). It will be helpful to exclude candidate therapeutic agents that are not effective at an early stage of drug development.

In acute toxicity assessment, percentage mortality and immobility are calculated. From that LC_{50} of TCEAE leaves was found to be 6.31mg/ l denotes its safe and non-toxic nature. (Previous report stated that LD_{50} of the ethanolic extract of the leaves in rats found to be1300mg/kgbw/p.o).

We screened the cardio protective effect of TCEAE on the myogenic heart of *D.magna*. The heartbeat of control, lactose induced, TCEAE 20,40,60,80 µg/ml and metoprolol 20, 25µg/ ml treated were found to be 190.9± 0.86, 93.2± 1.19, 138.6± 1.07, 159.6± 1.12, 188.8± 0.77, and 190.47± 0.54, and 176.6± 1.17, 189.7± 0.70 bpm respectively. The results were encouraging that it is comparable to that of the standard drug metoprolol. The results was statistically significant ($p<0.05$)

Therefore the above studies proved that the TCEAE of the leaves of *T.cucumerina* is therapeutically effective agent in arrhythmic heart without toxicity and its effectiveness comparable to currently available therapeutic agent metoprolol, a class of β-blocker available for the treatment of cardiac arrhythmias. This research provide a pathway to a newer drug lead or herbal formulation without arrhythmogenic potency.

From this investigation it is confirmed that TCEAE leaves is a cheap, easily accessible, safe and additive to conventional treatment of hypertension. The activity may be due to the presence of quercetin and other polyphenols, triterpenoids and high trace element potassium and calcim present in the TCEAE leaves.

It was reported that leaves of *T.cucumeriana* used for wound healing in folk medicine. So we have planned to provide scientific background of this ethnomedical claims. So the restoration of damaged tissue as closely as possible to its normal state mainly studied. Our study confirms the traditiional use of the leaves *T.cucumerina* for wound healing . Wound healing evaluated by *ex-vivo* porcine skin wound healing model (PSWHM). Architecture of pig skin is closer to human (Bollen Peter JA et al, 1999, Laber et al, 2002). Porcine model is an excellent tool for the evaluation of therapeutic agent meant for wound healing (Sullivan TP et al, 2001). Epidermal migration was measured (Nayak S, 2006).

Epidermal migration or keratinocyte migration distances from the edges of each wound were measured, normalized with the PBS control group and expressed as mean%. The result clearly showed TCEAE (3%) promoted statistically significant ($p<0.05$) Wound healing effect is comparable to the standard drug mupirocin.

Wound healing activity of TCEAE of the leaves may be due to its high amount of quercetin and other flavanoids, triterpenoids constituents. Both of them known to have astringent property which is responsible for wound contraction and increased rate of epithelialisation along with the supportive anti-microbial activity (Nayak S, 2006). More over trace elements supports wound healing property as essential trace mineral are required for cellular growth and replication (Pereira CE, 1998). From the reports it is assumed that the higher trace elements content reported in EDS analysis might have also enhance the wound healing property.

Here we want to emphasise the traditional use of the aerial partsfor the treatment of diabetes and the several supportive scientific research of this claim as antidiabetic. (Arawwawla LD. *et al*., 2009)The common complication of diabetic patient is wound as a

adverse effect which are an enormous burden on the health care system, both in terms of cost and intensity of care required. Conclusively our study showed significant enhancement of wound repair and therefore can be beneficially, safely used as auxiallary therapy in diabetic patient with foot ulcers in addition to the other available treatment.

CHAPTER-7

CONCLUSION

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CONCLUSION

The present study highlights the pharmacognostical, phytochemical and potential cardioprotective action against lactose induced arrrthymic heart of *D.magna*, more effective therapeutically comparable to the synthetic counterpart, without toxicity of the ethyl acetate extract of the leaves of *Trichosanthes cucumerina* Linn belonging to the family Cucurbitaceae, a widely, easily available edible plant. Ethno medical information revealed that it was used in various ailments for long time all over the world. Traditional uses includes wide panel of disease. Diabetes, **wound**, , skin diseases like dermatitis, alopecia, liver congestion etc., and used as antiinflammatroy, **cardiotonic,** antipyretic, anthelmintic, diuretic etc. So it was also investigated for *ex vivo* wound healing activity to provide scientific grounds of its ethno medical claims. The tremendous economic potentiality of this cash crop remains neglected by the scientists, technologists, physician, traders, administrators, policy makers, farmers etc.

The morphological evaluation showed the adherence of general character to the family.

Detailed microscopical characters of the leaves showed the usual leaf anatomical characters, vascular bundle, numerous multicellular uniseriate glandular and non glandular covering trichomes on both epidermis**,** collenchyma under the adaxial and abaxial epidermis and single layer of palisade cells, spongy parenchyma in the lamina. Petiole is triangular in T.S with two humps containing vascular strands and a ring of vascular bundles. Below the epidermis a layer of collenchyma is

present. Microscopic schedules (Vein islet and termination numbers, stomatal number and index, palisade ratio), physicochemical parameters(Ash values, extractive values, LOD) were evaluated and presented.

Scanning Electron Microscopy of midrib showed many folded appearance. No diagnostic feature and new kind of microcostituents not previously recognised and apparently simple structure which may be extremely complex was observed.

Preliminary phytochemical screening showed the presence of carbohydrates, proteins and amino acids, flavonoids, saponin, terpenoids, tannins and phytosterols. Fixed oil, alkaloids, volatile oil were found to be absent.

Total flavonoid content in terms of quercetin found as 29mg/g and total phenolic content 76.5 mg in terms of gallic acid.

Trace elements analysis by EDS showed the presence of Ca, K, Mg, and P, Cl.

HPTLC profile of TCEAE of the leaves was studied. The presence of **apigenin and quercetin** was identified and quantified as 0.77 and 20% respectively.

Epidemiological studies show an inverse correlation between dietary flavonoid intake and mortality from coronary heart disease (CHD) which is explained in part by the inhibition of low density lipoprotein oxidation and reduced platelet aggregability(Cook NC and Samman S .1996)

The most frequently studied flavonoid, quercetin, has been shown to have biological properties consistent with its sparing effect on the cardiovascular system. Quercetin and other flavonoids have been shown to modify eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses), protect low-density lipoprotein

from oxidation (prevent atherosclerotic plaque formation), prevent platelet aggregation (antithrombic effects), and promote relaxation of cardiovascular smooth muscle (antihypertensive, antiarrhythmic effects).(Fromica JV and Regelson W 1995)

The **3R's** ethical principle (**R**eduction, **R**efinement, and **R**eplacement) was implemented that help to minimize harms to vertebrate animals used in science. In our study we used *D.magna* (water flea) as its genomic sequence shares most with humans and provided as an excellent model system that is relevant to studies of human cardiac disorder.

Acute toxicity assessment using daphnids showed **LC_{50}** of TCEAE of the leaves **6.31 mg/L** showing safe and non toxic (as in the case of reported ethanolic extract).

Prescreen of cardio protective activity against lactose induced arrhythmic myogenic heart of *D.magna* showed statistically significant($p<0.05$) dose dependent cardiac protection. TCEAE of the leaves may be therapeutically effective agent on arrhythmic heart without toxicity and its effectiveness comparable to currently available therapeutic agent metoprolol, a class of β-blocker available for the treatment of cardiac arrhythmias.

Mechanism of action: The lactose induced arrhythmia is due to its effect on Ion channel (K^+, Na^+, and Ca^{2+}) and cell signaling. So we assumed that the protective effect may be due to the reversal effect on ion channel and cell signalling of the myogenic heart of *D.magna* (this is not by osmotic effect of lactose, for e.g. through stretch receptors. Since this is not supported by the fact that neither glucose nor

galactose at the concentration had an effect on *D.magna* heart, nor by timings of the lactose inhibition and its reversal).

We also assumed that the cardio protection may be due do the presence of quercetin and apigenin and poly phenol content and high potassium level.

The results were encouraging that it is comparable to that of the standard drug metoprolol. This research provides a pathway to a newer drug lead or herbal formulation without arrhythmogenic potency.

Traditionally claimed wound healing activity was evaluated by using *ex-vivo* porcine ear wound healing model (PEWHM). TCEAE of the leaves (3%w/w) showed statistically significant wound healing effect which is comparable to the standard drug mupirocin which is the currently recommended drug for wounds and diabetic foot ulcer.

It may be suggested for treating various types of wounds in human beings. Development of wound healing agent from a natural drug is cheaper, safer and effective.

Further studies are needed for purification compounds in extract and to understand the complete mechanism of wound healing activity of *T.cucumerina* leaves.

Conclusively our study showed significant enhancement of wound repair and therefore can be beneficially, safely used as auxiallary therapy in diabetic patient with foot ulcers in addition to the other available treatment.

Hence the above investigation offers immerse scope for R & D landscape of cardio production and wound healing.

We recommend further investigations in animal model and clinical trials to confirm this potential therapeutic effect. These aspects remain to be studied.

Moreover the vast economic potentiality of this crop can be adequately exploited and can create employment opportunity to an agricultural worker throughout the year.

CHAPTER-8

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REFERENCES

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